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THE DETERMINATION OF COBALT IN FORAGE CROPS[1]

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ABSTRACT

A study was made of the nitroso-R-salt method for the determination of cobalt in forage crops. Wet ashing with nitric and perchloric acids was found to be the most satisfactory. Buffering of the solution of plant ash with 40 per cent ammonium citrate prior to the extraction of the cobalt with dithizone gave improved results. In addition this buffer was used in the development of the cobalt-nitroso-R-salt complex. The lack of precision experienced when bromine is used to discharge the colour of excess nitroso-R-salt was alleviated by the use of chlorine which is produced in situ with hydrochloric and nitric acids. Absorption spectra of nitroso-R-salt complexes of interfering elements are presented and 525 mμ is suggested as the wavelength for photometric estimation. The analysis of 129 samples of pure species of grasses and legumes is given indicating a species and variety difference in cobalt content. Generally grasses were found to contain less cobalt than legumes. A preliminary survey of the cobalt content of pastures in Ontario has shown that there are areas in this province where the forage material is quite low in cobalt.

It has been established that there are many areas of the world where animals grazing upon herbage of low cobalt content have become afflicted with a wasting disease. Because the disease is evidently due to a deficiency of cobalt in the forage crops, interest has been taken by many workers in the determination of the cobalt content of such crops. The amounts of cobalt reputed to be present in the forage materials are minute (0.03–0.1 p.p.m.) and most of the methods for determining this element quantitatively are unsuitable due to a lack of sensitivity. In addition, disagreement among collaborators analysing identical samples of forage crops by the same procedure raises doubt as to the reliability of the more sensitive methods now in use.

The ultimate purpose of the study presented in this paper was the analysis of a number of samples of forage crops to acquire some knowledge as to the status of cobalt in the forages grown in Ontario. Before this phase of the study was undertaken, an attempt was made to improve the most commonly used procedure involving the nitroso-R-salt reagent for the determination of minute amounts of cobalt. A modification of this method was developed and this improved procedure was used for the analysis of samples of pure species of forage crops, grown on the Ontario Agricultural College

[1] Contribution from the Department of Nutrition, Ontario Agricultural College, Guelph, Ont. This work formed a part of the thesis submitted by the senior author to the Ontario Agricultural College and the University of Toronto in partial fulfilment of the requirements for the degree of Master of Science in Agriculture.

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experimental plots, to determine to what extent the cobalt content of the forage varies with the species which comprise them. In addition, samples of pastures from various counties of Ontario were analysed as a tentative survey of the grazing areas of this province.

For the sake of convenience the results of the studies are presented in two parts, one dealing with the method of determining cobalt, the other with the cobalt content of pure species and samples of forages obtained from farms.

PART I. PROCEDURE FOR THE DETERMINATION OF COBALT IN FORAGE CROPS

EXPERIMENTAL AND DISCUSSION

Of the methods outlined for determination of cobalt by nitroso-R-salt (2, 5, 6, 8) that of Marston and Dewey (6) was found to be the most sensitive. The investigations reported in this part of the paper deal with the modification and adaptation of this method to routine analysis of plant material. Since it is desirable to discuss each step of the procedure in the light of findings of other workers, detailed reviews pertaining to the various steps are presented in the sections dealing with particular aspects of the method.

Early in this work it was found that there was considerable variation in the degree of colour development when the bromination procedure was employed for the discharge of the colour of the excess nitroso-R-salt. For this reason the study of the method began with the final steps of the procedure and modifications necessary to obtain precision and accuracy were introduced. Each previous step was then investigated and the measurement of the cobalt was made using the improved techniques. In this paper, however, the material is presented in the order in which the chemical determination is normally carried out.

Ashing Procedure

Following the work of Hiscox (4) who claimed that wet ashing with nitric and perchloric acids was the most efficient for cobalt determination, a comparison was made between that method and the nitric, perchloric and sulphuric acid method of Marston and Dewey (6).

Both methods were found to be equally efficient in extracting cobalt from the plant material and in the recovery of added cobalt. It was found, however, that excess sulphuric acid is difficult to remove and for this reason the digestion with nitric and perchloric acids only is to be preferred. In the latter procedure most of the excess acid can be fumed off readily, the ash taken into aqueous solution and adjusted to pH 8.0 with a minimum amount of ammonia water prior to extraction of cobalt from the solution of plant ash.

Extraction of Cobalt from Solutions of Plant Ash

Marston and Dewey employed a citrate-phosphate-borate buffer to adjust the pH of the ash solution to 8.3 before extracting cobalt with diphenylthiocarbazone (dithizone). With this procedure it was found that with certain samples a precipitation of slightly soluble salts occurred. In a procedure using nitrosocresol, outlined by Ellis and Thompson (3), 40 per cent ammonium citrate was used and this buffer system was investigated in the following experiment.

TABLE 1.—THE EXTRACTION OF COBALT BY DITHIZONE IN CARBON TETRACHLORIDE FROM SOLUTIONS CONTAINING IRON, NICKEL AND COPPER, AND BUFFERED WITH AMMONIUM CITRATE

Micrograms of Cobalt Added	Micrograms of Cobalt Recovered
0.50	0.50
0.50	0.47
1.00	0.98
1.00	1.00
2.00	2.05
2.00	1.97
3.00	3.00
3.00	2.95
4.00	3.95
4.00	4.00
5.00	5.05
5.00	4.93

The ashes from two samples which has previously caused precipitation, were adjusted to pH 8.0 by using 1 ml. of 40 per cent ammonium citrate for each gram of sample and titrating with 1 : 1 ammonia water to the required pH. The resulting solutions showed no evidence of precipitation for at least 24 hours.

Recovery experiments were made to determine if cobalt could be extracted quantitatively from solutions containing copper, iron and nickel when ammonium citrate buffer was employed. Solutions containing 0.5–5.0 micrograms of cobalt, 100 micrograms of copper, 500 micrograms of iron and 10 micrograms of nickel were adjusted to pH 8.0 with 40 per cent ammonium citrate and 1 : 1 ammonia water. The cobalt was extracted with dithizone in carbon tetrachloride and was determined colorimetrically with nitroso-R-salt by a modified procedure described in the last section of Part I of this paper.

The data given in Table I show that quantitative recovery of cobalt is possible from a solution containing cobalt, copper, iron and nickel when ammonium citrate is used as the buffer instead of the citrate-phosphate-borate buffer recommended by Marston and Dewey (6).

The procedure for ashing the cobalt dithizonate prior to colour development, was also investigated to determine if sulphuric and nitric acids were necessary to complete the digestion. It was found that digestion with 2 ml. of 60 per cent perchloric acid results in a clear colourless solution which leaves a white ash when the excess acid has been removed by heating on a hotplate. If the acid is fumed off gently there is no loss of cobalt. The last traces of acid should be removed, however, to facilitate the adjustment of the pH of the solution when the ash is dissolved in the subsequent steps of the determination.

Buffer for Colour Development

According to Marston and Dewey (6) the optimum range of pH for the formation of the red coloured complex of cobalt and nitroso-R-salt is pH 6 to 8. In the present work it was observed that precipitation occurred when the reactants were heated in the presence of the citrate-phosphate-borate buffer used by these workers for the maintenance of the required pH.

TABLE 2.—COMPARISON OF THE COBALT CONTENT OF FORAGE SAMPLES DETERMINED BY THE PROCEDURES USING THE CITRATE–PHOSPHATE–BORATE BUFFER AND THE AMMONIUM CITRATE BUFFER

Laboratory No. of Sample	Citrate–Phosphate–Borate Buffer	Ammonium Citrate Buffer
	p.p.m. Cobalt	p.p.m. Cobalt
13	0.090	0.098
	0.054	0.110
14	0.073	0.101
	0.040	0.098
15	0.052	0.068
	0.040	0.065
16	0.063	0.084
	0.032	0.082
18	0.066	0.064
	0.037	0.068
19	0.015	0.030
	0.032	0.034

As such precipitation may result in occlusion of cobalt and hence lower values when nitroso-R-salt is added, the use of ammonium citrate as a buffer was investigated. Six samples of forage material were analysed and a comparison of the efficiency of the two buffers in the development of the cobalt was made.

The duplicate values for each sample for each buffer system are given in Table 2. These data show that better precision and recovery of cobalt are obtained in the presence of the ammonium citrate buffer than with the citrate-phosphate-borate buffer.

Discharge of the Colour of the Nitroso-R-salt Reagent

The red cobalt complex is formed in the presence of an excess of the nitroso-R-salt reagent, the yellow colour of which persists with only slight modification after boiling in nitric acid. Marston and Dewey employed bromine to discharge this yellow colour, and removed the excess bromine by boiling the solution. When the method described was followed it was found that reproducible results were difficult to obtain, as the time of heating and boiling each had an effect on the efficiency with which the colour of the excess reagent was discharged. In addition these two factors appeared to contribute to the destruction of the red colour of the cobalt complex to a varying degree.

In view of the unsatisfactory results obtained with bromine, consideration was given to the use of chlorine as a decolorizing agent. Since chlorine does not have the reddish colour of bromine and is much less soluble, the colour due to residual chlorine would impart a considerably smaller error in the measurement of the colour of the cobalt complex.

Cobalt-nitroso-R-salt complexes of varying amounts of cobalt (vol. 4.2 ml.) were prepared in 50 ml. beakers according to the method of Marston

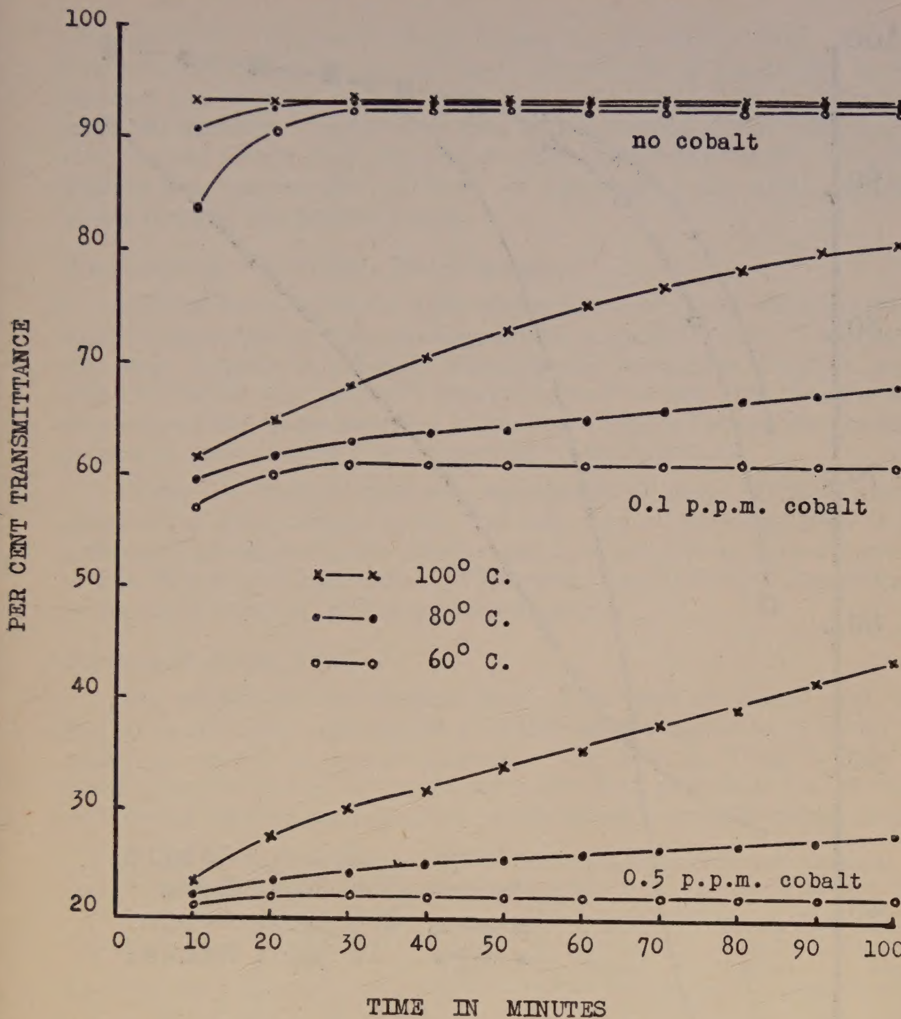

FIGURE 1. Effect of varying the temperature of the chlorination of nitroso-R-salt and cobalt-nitroso-R-salt complexes on the transmission of light at 420 mμ. Water used as 100 per cent transmission.

and Dewey (6). The beakers containing the solutions were placed for five minutes in a water bath maintained at a temperature of 60° C. to equalize the temperature of the solutions with that of the bath. Chlorine was produced in situ by adding 1 ml. of concentrated nitric acid and 2 ml. of concentrated hydrochloric acid to the contents in each beaker. The solutions were cooled and diluted to 10 ml.

The transmission of light by solutions containing no cobalt, 0.1 p.p.m. cobalt and 0.5 p.p.m. cobalt were determined in a Coleman Model 11 spectrophotometer at wavelength 420 mμ using 5 cm. cells and water as 100 per cent transmission. Figure 1 shows the effect of chlorine on the transmission of light by solutions of nitroso-R-salt, and cobalt-nitroso-R-salt complexes. At 80° C. and 100° C. the colour of the complex fades with

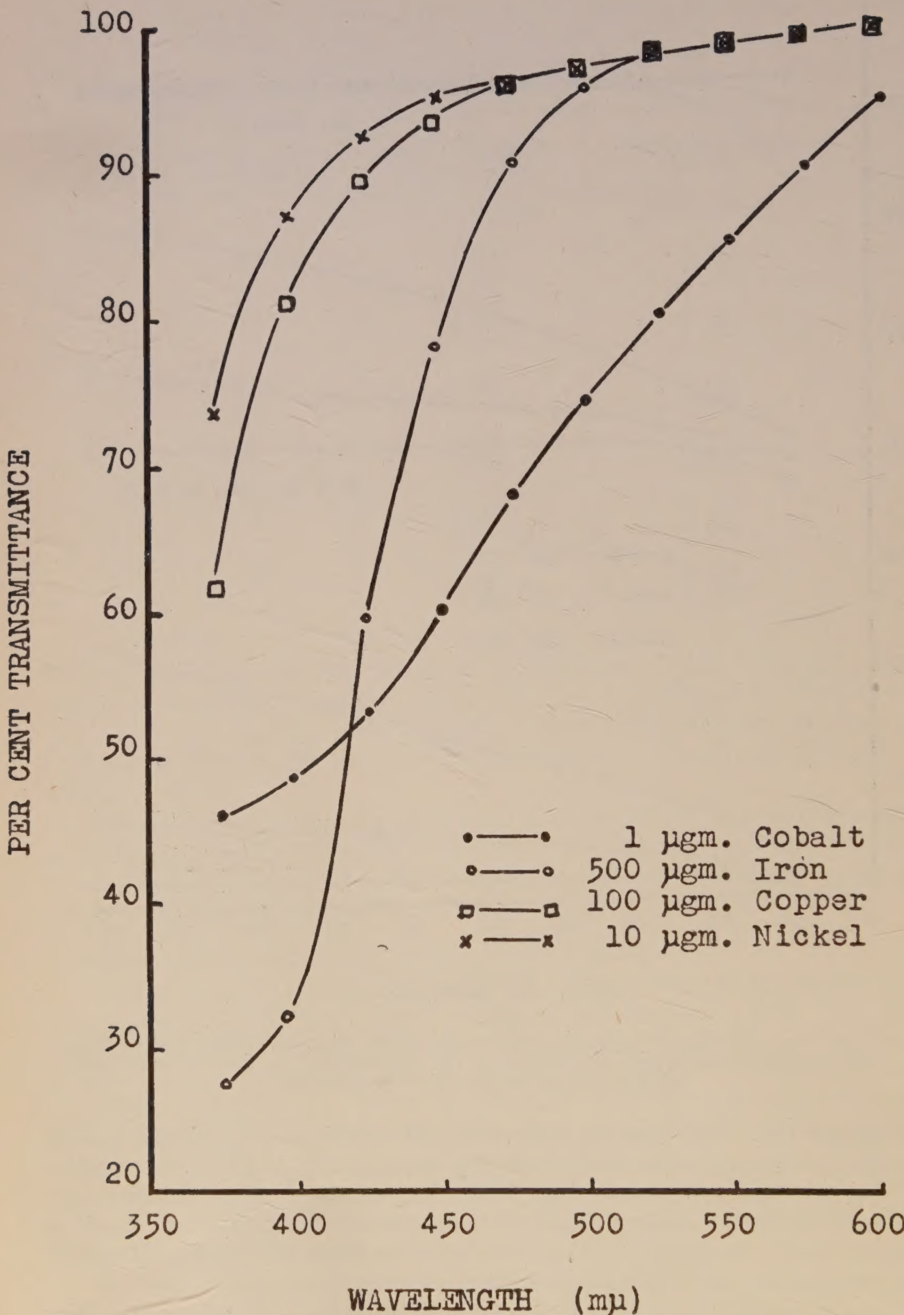

FIGURE 2. Absorption spectra of nitroso-R-salt complexes of cobalt, iron, copper and nickel. Water used as 100 per cent transmission.

time and this is reflected in the increasing transmission of light. At 60° C., however, the colour of the nitroso-R-salt reagent is discharged in 30–40 minutes and there is no apparent effect upon the cobalt complex for at least 100 minutes. From these data it was concluded that chlorine is an effective and satisfactory agent for decolorizing nitroso-R-salt. This modification was used in the procedure for determining the cobalt content of forage crops in the present study.

Wavelength for Colorimetric Determination

Various wavelengths for determining photometrically the absorption of light by the cobalt complex have appeared in the literature. To determine the most suitable wavelength, nitroso-R-salt complexes of cobalt, copper, iron and nickel were prepared separately and treated with chlorine in the manner outlined in the modified procedure. Figure 2 shows the absorption spectra of these treated complexes using water as a blank.

These data indicate that at a wavelength of about 525 mμ there is no interference from copper, nickel and iron, and at the same time there is sufficient absorption by the cobalt complex to provide an accurate measure of this element in forage crops. The use of this wavelength also makes the preliminary removal of copper unnecessary.

Recovery of Added Cobalt

The efficacy of the modifications which were developed was tested further in recovery experiments in which known amounts of cobalt were added to samples of forage material. The data in Table 3 show that satisfactory recoveries of cobalt were obtained and that the method is suitable for routine determination of the cobalt content of forage crops.

TABLE 3.—THE RECOVERY OF COBALT ADDED TO SAMPLES OF FORAGE MATERIAL

Sample number	Weight (grams)	Cobalt Content (μg.)	Cobalt Added (μg.)	Cobalt Found (μg.)	Cobalt Recovered (μg.)
1	10	0.58	0.5	1.11	0.53
2	10	0.47	0.5	0.94	0.47
8	10	0.52	0.5	0.97	0.45
10	10	0.44	0.5	1.00	0.56
24	10	0.33	0.5	0.75	0.42
25	10	0.45	0.5	0.95	0.50
24A	10	0.66	0.5	1.06	0.40
25A	10	0.50	0.5	0.95	0.45
143	10	0.67	0.5	1.12	0.45
144	10	0.62	0.5	1.17	0.55
18	10	0.83	1.0	1.93	1.10
21	10	0.80	1.0	1.92	1.12
32	10	1.09	1.0	2.00	0.91
33	10	0.84	1.0	1.77	0.93
6	10	0.93	1.0	2.09	1.16
7	10	1.09	1.0	2.12	1.03
13	10	1.00	1.0	1.83	0.83
14	10	0.95	1.0	1.82	0.87
118	10	1.11	1.0	2.11	1.00
122	10	0.91	1.0	1.85	0.94

MODIFIED METHOD FOR THE DETERMINATION OF COBALT IN FORAGE CROPS

Reagents

(*a*) Nitric acid—C.P. 70 per cent sp. gr. 1.42.

(*b*) Perchloric acid—C.P. 60 per cent sp. gr. 1.54.

(*c*) Hydrochloric acid—C.P. 37 per cent sp. gr. 1.19.

(*d*) Hydrochloric acid 0.01N—Dilute 0.83 ml. of C.P. hydrochloric acid to one litre with glass distilled water. Store in a glass-stoppered bottle or dispensing burette.

(*e*) Glass distilled water—Redistil laboratory distilled water from an all glass still and retain in all glass flasks or washbottles.

(*f*) Carbon tetrachloride—Distil technical grade carbon tetrachloride over calcium oxide.

(*g*) Ammonium hydroxide 1 : 1—Distil 500 ml. of ammonium hydroxide (28 per cent sp. gr. 0.90) into 500 ml. of glass distilled water. Retain in a glass-stoppered flask.

(*h*) Nitroso-R-salt 0.2 per cent—British Drug Houses reagent is used. Dissolve 2 grams of the nitroso-R-salt in 1 litre of glass distilled water. Filter through a No. 1 Whatman filter paper into a glass-stoppered bottle or dispensing burette. The solution is stable for at least a year.

(*i*) Ammonium citrate 40 per cent—Dissolve 400 grams of lead-free citric acid in 400 ml. of glass distilled water. Place the container in a cold water bath. Distil 450 ml. of concentrated ammonium hydroxide into this solution. Adjust the pH to 8.0 with 1 : 1 ammonium hydroxide. Dilute to one litre with glass distilled water. To remove any cobalt present extract with 50 ml. portions of dithizone solution in carbon tetrachloride until the aqueous phase stays orange coloured and the carbon tetrachloride is green in colour. Then extract the solution with carbon tetrachloride until the orange colour is removed. Filter the ammonium citrate solution through a No. 1 Whatman filter paper to remove the last traces of carbon tetrachloride.

(*j*) Dithizone 0.05 per cent in carbon tetrachloride—Eastman Kodak dithizone (diphenylthiocarbazone) is used. Purify the reagent by the method of Ellis & Thompson (3).

Procedure

To 10 grams of dry plant tissue in a 300 ml. Kjeldahl flask add 70 ml. of concentrated nitric acid and permit the mixture to stand over-night. Add a further 10 ml. of concentrated nitric acid and heat on a low flame until the sample appears as a jelly-like mass. Remove from the flame and add 20 ml. of 60 per cent perchloric acid. Carefully mix by rotating the flask. Return the flask to the burner and heat slowly until the appearance of white fumes of perchloric acid. Increase the heat and evaporate to a volume of 10–15 ml. Cool and dilute with 30 ml. of glass distilled water.

Transfer the contents to a 250 ml. beaker and evaporate to 2–3 ml. on a hotplate. Add 10 ml. of glass distilled water and filter through a No. 1 Whatman filter paper into another 250 ml. beaker. Wash the residue with two 10 ml. quantities of glass distilled water and dilute the filtrate to about 80 ml.

To the filtrate or suitable aliquot add 10 ml. of 40 per cent ammonium citrate. Adjust the pH to 8.0 with 1 : 1 ammonium hydroxide, using glass electrode. Transfer the solution to a 250 ml. separatory funnel. Add 10 ml. of 0.05 per cent dithizone in carbon tetrachloride. Shake for 3 minutes. Draw off the carbon tetrachloride phase into a 250 ml. separatory funnel containing 100 ml. of glass distilled water, acidified with 1–2 drops of concentrated hydrochloric acid. If necessary, repeat the

extractions with further 5 ml. quantities of dithizone solution. The extraction is complete when the aqueous phase remains orange and the carbon tetrachloride extract remains predominantly green in colour. Add 5 ml. of carbon tetrachloride to the aqueous phase. Shake for 1 minute. Add the carbon tetrachloride to the dithizone extracts. Repeat the extraction with a further 5 ml. of carbon tetrachloride and add to the combined extracts.

The dithizone extracts contain all of the cobalt. Transfer these extracts to a 50 ml. beaker. Add 2 ml. of 60 per cent perchloric acid. Cover with a watch-glass and reflux at a medium temperature on a hotplate until the carbon tetrachloride is evaporated and the dithizonates are completely digested. This results in a colourless solution. Remove the watch-glass and slowly evaporate off all perchloric acid. If free acid remains it will interfere with subsequent steps where pH adjustment is important.

After cooling, dissolve the cobalt-dithizonate ash in 1 ml. of 0.01 N hydrochloric acid. Add 1 ml. of 40 per cent ammonium citrate and 1 ml. of 0.2 per cent nitroso-R-salt. Cover with a watch-glass and place in a water-bath at 60° C. for 5 minutes. Slowly add 1 ml. of concentrated nitric acid to the solution rotating the solution to mix well. In a similar manner add 2 ml. of concentrated hydrochloric acid. Keep in the water-bath for a further 40 minutes, covered with a watch-glass. Remove from the bath and permit the solution to cool. Transfer the contents to a 10 ml. volumetric flask. Rinse out the beaker with two 1 ml. quantities of distilled water. Dilute the contents of the flasks almost to volume. When the solutions in the flasks have come to room temperature, dilute to volume and mix. Determine the per cent light transmitted by each solution in a 5 cm. cell, using a Coleman Model 11 spectrophotometer at wavelength 525 mμ. A blank solution on all reagents is used as 100 per cent transmission of light.

Prepare a standard curve by treating 1 ml. solutions containing 0.5 to 5 micrograms of cobalt in the manner described above. Prepare the blank by treating 1 ml. of 0.01 N hydrochloric acid in a similar manner and use the blank as 100 per cent transmission of light. One microgram of cobalt in 10 ml. of coloured solution transmits approximately 80 per cent of the incident light. A straight-line relationship exists from 0.5 to 5.0 micrograms of cobalt per 10 ml. of solution when the concentration of cobalt is plotted against the logarithms of transmitted light.

PART II. ANALYSES OF FORAGE CROPS

The cobalt content of 129 pure species of forage crops and 53 pasture mixtures grown in Ontario was determined employing the modified method outlined in the previous section. The averages of duplicate determinations are presented in Tables 4, 5 and 6.

Pure Species

Samples of pure species of grasses and legumes were obtained from the experimental plots at the Ontario Agricultural College. The seed for these crops originated in many parts of the world and was propagated by the

TABLE 4.—THE COBALT CONTENT OF PURE SPECIES OF GRASSES GROWN AT THE ONTARIO AGRICULTURAL COLLEGE

Species	Variety	Source of Seed	Cobalt p.p.m.
Brome Grass	N. Caucasus	U.S.S.R.	.03
	O.A.C. No. 1	Ont. Agricultural College	.05
	G. E. Lee	Soldier, Iowa	.05
	Common	Canadian Commercial	.05
	Moscow	U.S.S.R.	.05
	Nebraska	Lincoln, Nebraska	.05
	Lincoln	Beltsville, Maryland	.05
	Dom. Forage Lab. S. 1651	Saskatoon, Saskatchewan	.05
Tall Oat	Odenwalder	Frankfurt, Germany	.05
	M.I. 4523	Elsberry, Montana	.06
Agropyron	Elongatum 1978-47	Waterloo, Nebraska	.05
	Intermedium 3858-47	Waterloo, Nebraska	.07
Timothy	Otofte	Roskilde, Denmark	.05
	Hohenheimer	Frankfurt, Germany	.06
	O.A.C. Pasture	Ont. Agricultural College	.06
	O.A.C. No. 1	Ont. Agricultural College	.06
	Ohio	Wooster, Ohio	.07
	Svalof	Alberta	.07
	Milton	Macdonald College, Quebec	.07
	Marietta	Beltsville, Maryland	.08
	Climax	Central Expt. Farm, Ottawa	.08
	Kampe II	Sweden	.10
	Common	Canadian Commercial	.10
Orchard Grass	Brage	Goteborg, Sweden	.06
	Roskilde I	Roskilde, Denmark	.06
	Hercules	Central Expt. Farm, Ottawa	.06
	O.A.C. No. 1	Ont. Agricultural College	.07
	Tardus II	Sweden	.08
	F.C. 23097	Beltsville, Maryland	.08
	Wisconsin No. 52	Madison, Wisconsin	.09
	Common	Canadian Commercial	.09
	Weihenstephan	Germany	.10
Red Top	Oberhaunstadter	Frankfurt, Germany	.05
	O.A.C. Common	Ont. Agricultural College	.06
	Reton	Ont. Agricultural College	.07
	Common	Canadian Commercial	.09
Reed Canary Grass	Ioreed	Beltsville, Maryland	.05
	O.A.C. Strain	Ont. Agricultural College	.07
	Ioreed	Ames, Iowa	.08
	Superior	Corvallis, Oregon	.09
	Common	Canadian Commercial	.10
	O.A.C. No. 1	Ont. Agricultural College	.11
Meadow Foxtail	Weihenstephan	Germany	.08
Canada Blue Grass	Canon	Ont. Agricultural College	.08
	F.C. 23521	Beltsville, Maryland	.08
	Common	Canadian Commercial	.10
Meadow Fescue	No. 1449—F.C.—23263	Beltsville, Maryland	.07
	Suiter Strain	Beltsville, Maryland	.08
	Loken	Norway	.08
	Kentucky 31-47	Waterloo, Nebraska	.10
	Sidig	Roskilde, Denmark	.10
	Ensign	Central Expt. Farm, Ottawa	.11

TABLE 4.—THE COBALT CONTENT OF PURE SPECIES OF GRASSES GROWN AT THE ONTARIO AGRICULTURAL COLLEGE—*Concluded*

Species	Variety	Source of Seed	Cobalt p.p.m.
Meadow Fescue—*con.*	Common	Canadian Commercial	.11
	Steinacher	Frankfurt, Germany	.18
Red Fescue	Steinacher	Frankfurt, Germany	.09
	Otofte	Roskilde, Denmark	.09
	O.A.C. No. 1	Ont. Agricultural College	.11
	Wisconsin No. 84	Madison, Wisconsin	.12
	Duraturf	Central Expt. Farm, Ottawa	.13
	Reptans	Sweden	.15
	Weihenstephan	Weinstephan, Germany	.16
	Common	Canadian Commercial	.18
Perennial Rye	Peron	Ont. Agricultural College	.12
	Common	Canadian Commercial	.15
	Victory	Sweden	.17
Kentucky Blue Grass	Primo	Sweden	.14
	Common	Canadian Commercial	.17
	O.A.C. No. 1	Ont. Agricultural College	.19
	(Unknown)	Germany	.25

Department of Field Husbandry. The cobalt content of the grasses and of the legumes is presented in Tables 4 and 5, respectively. These data show that there is considerable variation in the cobalt content of different species grown on the same soil. Generally, grasses were found to have a lower cobalt content than legumes.

The amount of cobalt in grasses was found to vary with the species and the data in Table 4 are in general agreement with the findings of Beeson *et al.* (1). Of the grasses analysed, Kentucky blue grass contained, on the average, the largest amounts, 0.14–0.25 p.p.m., and brome grass the smallest amounts, 0.03 to 0.05 p.p.m. In addition there was a wide range among varieties of the same species. This was particularly evident among the fescues.

Various species of legumes exhibited marked differences in cobalt content, some of the species containing no more than the average of the grasses. The white clovers were found to have the highest amounts, varying from 0.17 p.p.m. to over 0.2 p.p.m., followed in order by the trefoils, red clovers, vetches, alfalfas and sweet clovers. Variety differences were also evident, among the legumes, as shown in Table 5.

The present data are not sufficiently extensive to identify any particular species of forage as being characteristically deficient in cobalt. It is recognized that at least two factors, composition of the soil and genetic factors in the various species of forage, may have an influence on the cobalt content of the plants. The data, however, indicate that, when a pasture does not maintain the health of ruminants by virtue of its low cobalt content, the species composition of the pasture is a factor which should be considered as well as a possible deficiency of available cobalt in the soil.

TABLE 5.—THE COBALT CONTENT OF PURE SPECIES OF LEGUMES GROWN AT THE ONTARIO AGRICULTURAL COLLEGE

Species	Variety	Source of Seed	Cobalt p.p.m.
Sweet Clover	White Common	Canadian Commercial	.06
	Yellow Common	Canadian Commercial	.07
Alfalfa	Ferax	Alberta	.05
	Wurzburg	Frankfurt, Germany	.06
	Canauto	Central Expt. Farm, Ottawa	.06
	China	Kansu Province, China	.06
	Williamsburg	Virginia	.07
	O.A.C. No. 1	Ont. Agricultural College	.07
	Frankische	Frankfurt, Germany	.07
	Ranger F.C. 23409	Beltsville, Maryland	.07
	Weihenstephan	Germany	.07
	Viking	Central Expt. Farm, Ottawa	.07
	Buffalo F.C. 23345	Beltsville, Maryland	.07
	Grimm	Common Variety	.07
	O.A.C. Strain	Ont. Agricultural College	.08
	Atlantic F.C. 23413	Beltsville, Maryland	.10
	Common	Canadian Commercial	.15
Vetch	Lathyrus Venosus	Grand Rapids, Michigan	.08
	Sainfoin Common	Central Expt. Farm, Ottawa	.12
Red Clover	49–18 Merker	Goteborg, Sweden	.07
	Molstad	Norway	.07
	Altaswede	Alberta	.07
	Resistanta	Sweden	.09
	Emerson	Iowa	.09
	Redon	Ont. Agricultural College	.10
	Macdonald College Late	Quebec	.10
	Emerson F.C. 23412	Beltsville, Maryland	.10
	Leon	Ont. Agricultural College	.11
	Otofte I	Roskilde, Denmark	.11
	Dollard	Macdonald College, Quebec	.11
	Weihenstephan	Sweden	.12
	F.C. 23274	Madison, Wisconsin	.13
	Double Cut	Canadian Commercial	.15
	Single Cut	Canadian Commercial	.16
	Lembkes	Frankfurt, Germany	.16
Hungarian Clover	—	Beltsville, Maryland	.13
Birdsfoot Trefoil	Empire	Ithaca, New York	.12
	V 102 Cornell	Ithaca, New York	.13
	E 501 Cornell	Ithaca, New York	.13
	B.N. 5489	Beltsville, Maryland	.13
	Bunker	Ithaca, New York	.15
	E 495 Cornell	Ithaca, New York	.16
	E 493 Cornell	Ithaca, New York	.17
	Kaellingstand	Roskilde, Denmark	.18
	E 491 Cornell	Ithaca, New York	.18
	E 494 Cornell	Ithaca, New York	.21
Alsike Clover	Mixture—Selected	Ont. Agricultural College	.17
	Common	Canadian Commercial	.23
	Alon	Ont. Agricultural College	.25
White Clover	O.A.C. Improved	Ont. Agricultural College	.16
	Otofte I.K. & V.	Roskilde, Denmark	.17
	Weihenstephan	Germany	.19
	Lodi-Otofte I.K. & V.	Roskilde, Denmark	.20
	Duron	Ont. Agricultural College	.21
	Robusta	Sweden	.21
	Oregon F.C. 23633	Beltsville, Maryland	.27
	Common	Canadian Commercial	.29
Ladino Clover	O.A.C. Improved	Ont. Agricultural College	.21
	F.C. 23040	Beltsville, Maryland	.22
	Common	Canadian Commercial	.22

TABLE 6.—THE COBALT CONTENT OF PASTURE FORAGE IN THE PROVINCE OF ONTARIO

Farm Number	Number of Samples	County	Cobalt Content of Forage p.p.m.
1	6	Ontario	0.05 0.05 0.05 0.06 0.06 0.07
2	6	York	0.04 0.04 0.04 0.05 0.05 0.06
3	4	Oxford	0.06 0.06 0.06 0.08
4	4	Welland	0.09 0.09 0.10 0.14
5	3	Stormont	0.09 0.10 0.10
6	2	Stormont	0.05 0.09
7	2	Carleton	0.04 0.05
8	2	Glengarry	0.05 0.06
9	2	Perth	0.10 0.12
10	2	Perth	0.07 0.09
11	2	Huron	0.06 0.07
12	2	Huron	0.10 0.11
13	2	Brant	0.27 0.33
14	2	Bruce	0.05 0.10
15	2	North Simcoe	0.06 0.12
16	2	North Simcoe	0.06 0.07
17	2	Grey	0.09 0.09
18	2	Wellington	0.03 0.07
19	2	South Simcoe	0.07 0.14
20	2	Dufferin	0.04 0.06

Pasture Mixtures

The cobalt content of samples of pastures from farms in various counties has been determined and the results are presented in Table 6. The samples from each farm represent the herbage grown in different areas of the farm.

McNaught (8) concluded that pastures containing on the average less than 0.07 p.p.m. of cobalt are unhealthy for sheep, while below 0.04 p.p.m. of cobalt, the pastures are unhealthy for cattle. On the basis of these criteria it is evident from the data of Table 6 that there are areas in Ontario in which pasture herbage is deficient in cobalt from the viewpoint of requirements of sheep and cattle. On some farms samples from different fields varied widely in their cobalt content. In these cases it was noted that the species composition of the pasture varied markedly and may have accounted for a major portion of the variation in cobalt of the forage.

In general, these preliminary findings support the report (7), based on therapeutic cobalt treatment of unhealthy cattle, that there are areas in Ontario in which the forage material is deficient in cobalt. Further studies are necessary, however, to establish the extent to which this deficiency exists in this province.

SUMMARY

A study was made of the nitroso-R-salt method for the determination of cobalt in forage crops. Several modifications were introduced increasing the accuracy and precision of the method.

Wet ashing with nitric and perchloric acids was found to be more suitable than with nitric, perchloric and sulphuric acids. Ammonium citrate gave satisfactory buffering of the plant ash solution prior to the extraction and concentration of cobalt with dithizone. Precipitation of slightly soluble salts which took place with the citrate-phosphate-borate buffer of the original method, did not occur in the presence of the ammonium citrate. This buffer also gave better results in the part of the procedure where the cobalt-nitroso-R-salt complex is developed.

Hydrochloric and nitric acids were used to produce chlorine in situ for the discharge of the colour of excess nitroso-R-salt reagent. Complete discharge of colour was accomplished by heating the chlorinated solution for 40 minutes in a water bath at 60° C. The colour of the cobalt-nitroso-R-salt complex was not affected under these conditions and this procedure was found to be more satisfactory than the treatment with bromine.

A study of the absorption spectra of the nitroso-R-salt complexes of cobalt and interfering metals suggests that 525 mμ is a suitable wavelength for colorimetric comparison, making the preliminary separation of copper unnecessary and providing sufficient absorption by the cobalt complex to give an accurate measure of the content of this mineral in forage material.

The modified procedure was used for the determination of the cobalt content of pure species of grasses and clovers grown at the Ontario Agricultural College. The data show that there is considerable variation in the cobalt content of different species grown on the same soil. In addition there was a wide range among varieties of some of the species. Generally the grasses were found to contain less cobalt than the legumes.

A preliminary survey of the cobalt content of pastures on Ontario farms has shown that there are areas in this province where the forage material is quite low in cobalt.

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NITROGEN CONSTITUENTS OF BURLEY TOBACCO RESULTING FROM AMMONIUM AND NITRATE NUTRITION

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ABSTRACT

Samples of top and bottom leaves and stalks of burley tobacco plants grown in sand, with a constant supply of nitrogen nutrient containing 0,33, 66 and 100 per cent as ammonium ions and the remainder as nitrate ions, were analysed for protein and the soluble nitrogen constituents: nitrates, nicotine, ammonia and amides.

High concentrations of protein and nicotine are associated with high ammonium nutrition, maximums occurring when plants received all or two-thirds of the nutrient nitrogen as ammonium ion. The storage of ammonia and nitrate in the tissues increases progressively in proportion to their respective nutrient supply. The maximum storage of nitrate nitrogen is several times that of ammonia nitrogen. Amides accumulate increasingly with the ammonium nutrient supply. Especially in the stalks and upper leaves of plants, the contents of both the total soluble nitrogen and the insoluble (protein) nitrogen display increasing trends as the proportion of ammonium nutrient is increased.

In Canada, fertilizer recommendations for tobacco stipulate that one-quarter of the nitrogen in commercial fertilizers must be in the nitrate form and the balance in standard water-soluble materials. Since the form of nitrogen applied in fertilizers is so important in ensuring early growth, high yield and desirable quality of tobacco crops, a knowledge of plant nitrogen constituents resulting from the two common nutrient forms of nitrogen is of fundamental importance.

The effect of the form of the nitrogen nutrient on plant composition has been studied rather intensively since about 1930. Of primary interest is the fact that considerable amounts of nitrate and ammonium nitrogen have been found to be absorbed by various plants and stored apparently without undergoing chemical change (1, 4, 5, 6, 12, 13, 14, 15, 16). The composition of tobacco plant material grown in sand cultures containing different proportions of ammonium and nitrate nitrogen nutrients has been studied by Vickery *et al.* (16), McEvoy (7), and Evans and Weeks (4). Earlier investigations with other plants include those of Tiedjens and Robbins (14), and Clark (1, 2).

McEvoy (7) found that plant growth and the contents of potassium, magnesium and calcium increased, while the total nitrogen and phosphorus decreased as the proportion of nitrate ions increased in the nutrient medium supplied to two burley tobacco varieties. He also found that a high supply of ammonium ions in the nutrient solutions produced magnesium deficiency symptoms of plants. The present paper is a continuation of McEvoy's study (7) and deals with various nitrogen constituents of leaves and stalks samples from the same sand-cultured plants.

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EXPERIMENTAL MATERIALS AND METHODS

The proportions of ammonium and nitrate ions were varied in four treatments for which McEvoy (7) found the following green weight yields per plant:

TABLE 1.—GREEN WEIGHT YIELDS PER PLANT (AFTER MCEVOY)

Treatment No.	Proportion of Total N-Nutrient		Green Yield of Burley Varieties	
	NH_4—N %	NO_3—N %	Harrow Velvet gm./plant	Kelley gm./plant
1	0	100	766.9	722.2
2	33	66	839.3	745.9
3	66	33	626.1	568.8
4	100	0	262.0	240.2

The green samples representing a definite weight of about 200 grams of top leaves, bottom leaves and stalks were preserved in a frozen state at $-7°$ to $-12°$ C. as suggested by Nightingale *et al.* (9). The top and bottom leaves represented leaves of the upper and lower half positions on plants, respectively.

Moisture was determined by drying the green material at 70° C. for 40 hours. All other analyses were conducted from extracts or extracted residues of the frozen samples. In the preparation of extracts and residues, the juice of the frozen material was squeezed out in a Carver press by 15,000 to 20,000 pounds pressure per square inch and made up to about 225 ml. by three hot water washings and squeezings of the press-residue. The residue was further extracted by boiling with 150 ml. of distilled water for five minutes, filtered and washed to about 225 ml. of filtrate. The combined extracted juices, made up to 500 ml. and preserved with a layer of toluene, were analysed for soluble N, nitrate, nicotine, ammonia and amide N fractions. The residue, dried at 70° C. for 24 hours, represented the insoluble dry matter on which the protein N was determined.

In addition, nicotine was determined in air-dried tissue samples. The methods of Vickery, Pucher and co-workers were followed in the analyses for total nitrogen (11), ammonia (17), amide (17, 18) and nitrate (19) nitrogen fractions. Nicotine was determined by the silicotungstic acid method (8) and protein by the Gunning method (10).

RESULTS

Results were obtained from samples of two burley varieties, viz., Harrow Velvet and Kelley, but because of the similarity of trends of the various nitrogen constituents the complete analytical data for only one variety, viz., Harrow Velvet, are presented in Table 2. However, in subsequent smaller tables, pertinent additional data are shown for both varieties. The data are expressed in parts per 1000 of green tissue weight or as otherwise stated.

Dry Matter and Water

The content of insoluble dry matter exceeded that of soluble dry matter in every instance (Table 2). The highest water content was found in the

TABLE 2.—NITROGEN DATA OBTAINED FROM ANALYSIS OF WATER EXTRACTS AND RESIDUES OF BURLEY TOBACCO (HARROW VELVET) IN PARTS PER 1000 OF GREEN MATERIAL

	Top leaves				Bottom leaves				Stalks			
Nutrient N — % as NH_4 ion	0	33.3	66.6	100	0	33.3	66.6	100	0	33.3	66.6	100
— % as NO_3 ion	100	66.6	33.3	0	100	66.6	33.3	0	100	66.6	33.3	0
Dry matter—Total	94.3	105.0	128.0	102.3	83.3	80.0	92.0	73.3	85.0	131.7	117.8	86.3
Insoluble dry matter	53.5	67.5	73.9	53.5	54.0	45.3	52.2	41.7	54.7	78.7	80.9	63.0
Soluble dry matter	40.8	37.5	54.1	48.8	29.3	34.7	39.8	31.6	30.3	53.0	36.9	23.3
Nitrogen—Total	4.52	5.57	6.46	6.74	3.96	3.60	4.24	4.13	2.37	2.59	3.01	2.67
Insoluble (protein) N	2.38	3.54	4.14	4.18	2.21	1.85	2.37	2.03	.678	.754	.786	1.03
Soluble N	2.14	2.03	2.32	2.56	1.75	1.75	1.87	2.10	1.69	1.84	2.22	1.64
Nitrate N	1.09	.646	.284	.027	.965	.692	.328	.043	1.44	.784	.373	.064
Nicotine N	.080	.199	.197	.134	.121	.121	.158	.136	.026	.137	.076	.099
Ammonia N	.069	.083	.283	.477	.038	.066	.239	.757	.040	.099	.243	.373
Amide N	.064	.136	.139	.283	.063	.083	.131	.172	.154	.264	.370	.310

lower leaves of plants. A clearer picture of the water and dry matter contents is given by the ratio of water to solids for the two varieties, Harrow Velvet and Kelley, in Table 3 showing minimum ratios in the stalk and leaf tissues when ammonium ion supplied 33 and 66 per cent of the nitrogen nutrient, respectively.

TABLE 3.—WATER : SOLIDS RATIOS IN GREEN MATERIAL

Burley Variety	Harrow Velvet				Kelley			
% of Nutrient N as Ammonium Ion	0	33	66	100	0	33	66	100
Top leaves	9.60	8.52	6.81	8.80	9.13	8.26	7.77	9.87
Bottom leaves	11.00	11.50	9.87	12.70	12.51	11.50	10.91	14.39
Stalks	10.76	6.58	7.60	10.63	10.63	8.26	8.52	8.87

Nitrogen Constituents

The total nitrogen data of Table 2 are represented by the sum of the insoluble and soluble N fractions. A lower concentration of total nitrogen resulted in the leaves and stalks from high nitrate nutrition than from high ammonium. A greater content of insoluble than soluble nitrogen was found in the leaves but the reverse relationship was found in the stalks. In general, especially in the stalks and top leaves, the soluble, insoluble and total nitrogen fractions increased in concentration as the ammonium nutrient was increased to supply two-thirds or all of the nitrogen. Higher quantities were found in the top than in the bottom leaves.

The nitrate content accounted for as much as 50 per cent of the soluble nitrogen in the leaves and more than 50 per cent in the stalks when all the nitrogen nutrient was in the nitrate form. As the proportion of ammonium nitrogen was increased in the nutrient solutions, the storage of nitrate in leaves and stalks progressively decreased while that of ammonia progressively increased. The maximum concentration of nitrate was found in the stalks in most cases while the bottom leaves contained more than the top leaves. In treatment 4, ammonia was more concentrated in the bottom leaves than in either the top leaves or stalks. When all the nitrogen nutrient was supplied in the nitrate compared to the ammonium form, the concentration of nitrate nitrogen in the tissues was several times as much as that of ammonia nitrogen, viz. 4, 2 and $1\frac{1}{3}$ times as much in the stalks, top leaves and bottom leaves, respectively.

From the analyses of extracts (Table 2), maximum concentrations of nicotine in leaves and stalks seemed to be associated with a high proportion of ammonium or nitrate in the culture solution. However, a more regular trend for nicotine was obtained from the analysis of air-dried sample material shown in Table 4 for the two varieties, Harrow Velvet and Kelley.

In the dry leaf analysis of both burley varieties, the nicotine content increased progressively as the proportion of ammonium was increased to supply 66 per cent of the nitrogen nutrient. The maximum content occurred in the top leaves in treatment 3. With Harrow Velvet the bottom leaves contained more nicotine than the stalks but with the Kelley variety the reverse was found.

TABLE 4.—NICOTINE ANALYSIS OF DRIED TISSUE (EXPRESSED IN GRAMS PER 1000 OF GREEN MATERIAL)

Burley Variety	Harrow Velvet				Kelley			
% of Nutrient N as ammonium ion	0	33	66	100	0	33	66	100
Top leaves	.084	.139	.207	.100	.076	.106	.144	.092
Bottom leaves	.096	.099	.144	.127	.027	.044	.066	.049
Stalks	.043	.081	.093	.102	.054	.112	.106	.082

Amides displayed regular increasing trends in the leaves but in the stalks this trend was interrupted when ammonium ion supplied all the nitrogen nutrient. The concentration of amides was highest in the stalks and higher in the top leaves than in the bottom leaves. The protein nitrogen increased regularly in the stalks and top leaves only (Table 2). Somewhat similar trends in the top leaves of the two varieties of burley tobacco plants are shown for the protein (N $\times$ 6.25) percentage analysis of the dried extracted residues in Table 5.

TABLE 5.—PROTEIN (N $\times$ 6.25) PER CENT DRIED EXTRACTED RESIDUE

Burley Variety	Harrow Velvet				Kelley			
% of Nutrient N as ammonium ion	0	33	66	100	0	33	66	100
Top leaves	29.9	35.1	38.0	39.0	38.1	38.3	43.7	42.9
Bottom leaves	27.9	27.4	30.8	32.4	29.4	28.8	37.4	35.7
Stalks	8.5	6.4	6.5	10.9	6.2	5.2	7.3	—

In the top leaves of Harrow Velvet the protein content increased from 29.9 to 39.0 per cent of the insoluble matter. The protein content was invariably higher in the leaves of the variety Kelley, than Harrow Velvet.

DISCUSSION

Under the conditions of the experiment conducted in sand culture, high yields resulted from high nitrate nutrition and low yields from high ammonium nitrogen nutrition (Table 1). A similar but less pronounced effect was obtained under field conditions at Ottawa in fertilizer experiments (6). Although treatments 1 and 2 (Table 1) produced high plant yields which did not differ significantly (7), the concentration of nicotine and amides were particularly higher in the top leaves and stalks for the latter treatment. No satisfactory explanation can be given as to why higher nicotine contents were obtained in the extracts than in the air-dried material, especially for treatment 2. Treatments 3 and 4 each produced significantly smaller plants with high contents of ammonia, amides and protein similar to field grown plants at an immature stage of development (6). However, the unusually high content of ammonia in the bottom leaves appears to be characteristic of magnesium deficient plants when all the nitrogen nutrient is supplied as ammonium ion (4, 7).

The variation in the size of plants is therefore also an important factor in causing nitrogen composition differences. Another important factor, apparently, concerns the hydrogen ion concentrations of the nutrient solutions which were not reported by McEvoy (7). In this connection, Tiedjens and Robbins (14) found that culture solutions of pH 8 for ammonium ions and pH 4 to 7 for nitrate ions were optimum reactions for the growth of tomato plants. In addition, Evans and Weeks (4) found that culture solutions adjusted to pH 5.8 produced at harvest smaller and more immature tobacco plants from ammonium ions than from nitrate ions.

According to present knowledge of nitrogen nutrition of tobacco in sand culture, the observed differences in nitrogen composition may be caused by at least four interrelated factors, viz., the size of the plants, the maturity of the plants, the initial hydrogen ion concentration of the nutrient medium, and the form of nutrient nitrogen. However, under field culture, such differences would not be expected to occur in acid soils between pH 5 and 7 with conditions favourable for nitrification of ammonium ions. Nevertheless, the fact that most tobacco soils have an acid reaction below pH 7.0 would seem to favour the use of nitrate in tobacco fertilizers. Also, the use of ammonium nitrate would be expected to produce satisfactory growth of tobacco over a wider range of soil pH values than the use of ammonium sulphate as the only source of nitrogen in tobacco fertilizers.

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LARVAL NUTRITION IN *AGROTIS ORTHOGONIA* MORR. (Lepidoptera: Phalaenidae). A NEW REARING METHOD

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ABSTRACT

Whole wheat seedlings are shown to be far superior to cut wheat leaf as a laboratory food for the larvae of *Agrotis orthogonia* Morr. This superiority is attributed to the greater food, and especially nitrogen, intake which is permitted by the greater water content of the former. This higher water content results from the ability of the root hairs to continue to pick up water from a moist substratum. Green plant material is not essential to growth. Neither nutrient salts nor 2,4-D added to the water used to germinate seedlings had any significant effect on the growth rate of larvae to which these were fed. The significance of these facts in the field, and the original development to pest status by this species are briefly discussed.

Since *Agrotis orthogonia* Morr. established itself as a major pest of cereal crops in the drier regions of the prairies of North America in 1911, many workers have maintained populations of this insect through parts of its life cycle, principally the later larval stages, in the laboratory (7, 9, 11). This work has been conducted on varying scales, and with different objectives; development has usually been much slower than development in the field (17). Almost without exception the food materials mentioned in published accounts have been cut leaf material of a variety of plant species, both crop plants and weeds being investigated. This seems strange in view of the acknowledged subterranean habit of this species, at least for the greater part of its larval development, and it was this fact that led to the investigations with whole wheat seedlings reported here.

COMPARISON OF WHEAT LEAF WITH WHEAT SEEDLINGS

Method

Larvae were hatched from eggs as nearly as possible at the same time, by subjecting eggs containing mature embryos to warm moist conditions. On hatching the larvae were placed on the appropriate food material in glass vials, which were then maintained at controlled temperatures, and in some instances, humidities.

Wheat seedlings were germinated a few days previously on moist filter paper in Petri dishes kept in the dark. Except in a few instances, when a slowly feeding larva permitted considerable further development of a plant, larvae fed on whole wheat seedling material had no access to tissues in which chlorophyll had developed. Wheat leaf was cut in lengths of about $\frac{3}{4}''$ from plants grown in pots indoors. Plants ranged from a few inches to nearly a foot in height. Most of the larvae (200) were kept in $3''$ lengths of $1''$ Pyrex tubing, closed $\frac{3}{4}''$ from the lower end with a shallow plug of plaster of paris, and at the top with a cork having a piece of $\frac{1}{4}''$ glass tubing projecting $\frac{1}{4}''$ through its lower end. These rearing tubes were kept in batches of fifty standing upright in wet sand in galvanized iron trays in a controlled temperature cabinet at 75° F.

Twenty larvae were reared in vials with perforated metal caps, and twenty in vials loosely plugged with cotton. These containers were stored in glass dessicators containing solutions of salts for humidity control and were maintained at daytime temperatures of 68° F. and night temperatures (2200-0600 hr.) of 58° F.

On every second or every third day, or occasionally at longer intervals, faecal pellets and residual food were removed, fresh food was given, and the larva was weighed. In 1949, however, weights were recorded at weekly intervals only. Similar weights of the two foods used were given; larvae fed cut leaf always had a residue of uneaten food at the next meal, in some instances those fed seedlings were without food for a short time, roots, shoots, and seeds being entirely consumed before the next feeding. Mortality was recorded, but no record was kept of the dates of moulting. Sex was recorded on pupation, and the pupae were transferred to moist soil in cages. Individual records were not kept after pupation.

Results

The results of these experiments are summarized in Table 1 and Figure 1. On the basis of growth rate and weight at pupation, whole wheat seedling is clearly a far more suitable food material than wheat leaf, and green plant material is certainly not essential to development. Mortality was high in all batches. Much of this was due to handling; it was higher in 1950 when larvae were weighed every second or third day than it was in 1949 when they were only weighed once a week. Many of the dead larvae, particularly those raised at high humidities, had a diseased appearance. Thus, although larvae grew faster, and weighed more when reared at 70 per cent R.H. than at 50 per cent, mortality was greater at the higher humidity. In plaster bottomed tubes mortality was very variable; the humidity in these depended of course on ventilation.

The better development on wheat seedling is undoubtedly due to the increased rate of food and especially of nitrogen intake. That water content plays an important role in permitting this, however, it is evident from the figures in the last column and in lines 3 and 5 in Table 1. The

TABLE 1.—MEAN WEIGHTS IN MGM. OF *A. orthogonia* LARVAE FED ON WHEAT SEEDLINGS AND WHEAT LEAF UNDER VARIOUS MOISTURE CONDITIONS, 1949 AND 1950. PERCENTAGE MORTALITIES IN BRACKETS

	On wheat leaf		On seedling wheat		Ratio, mean wt. on seedling to mean wt. on leaf 30 days
	21 days	30 days	21 days	30 days	
1. In vials at 50% R.H.—20 larvae	13 (30)	45 (50)	27 (20)	85 (30)	1.88
2. In vials at 70% R.H.—20 larvae	20 (40)	75 (60)	36 (40)	140 (40)	1.87
3. Ratio, wt. at 70% R.H. to weight at 50% R.H.	1.54	1.67	1.33	1.65	—
4. In plaster-bottomed tubes—200 larvae	45 (36)	170 (42)	270 (27)	450 (31)	2.65
5. Ratio, wt. in plaster tubes to wt. at 70% R.H.	2.25	2.27	7.5	3.22	—

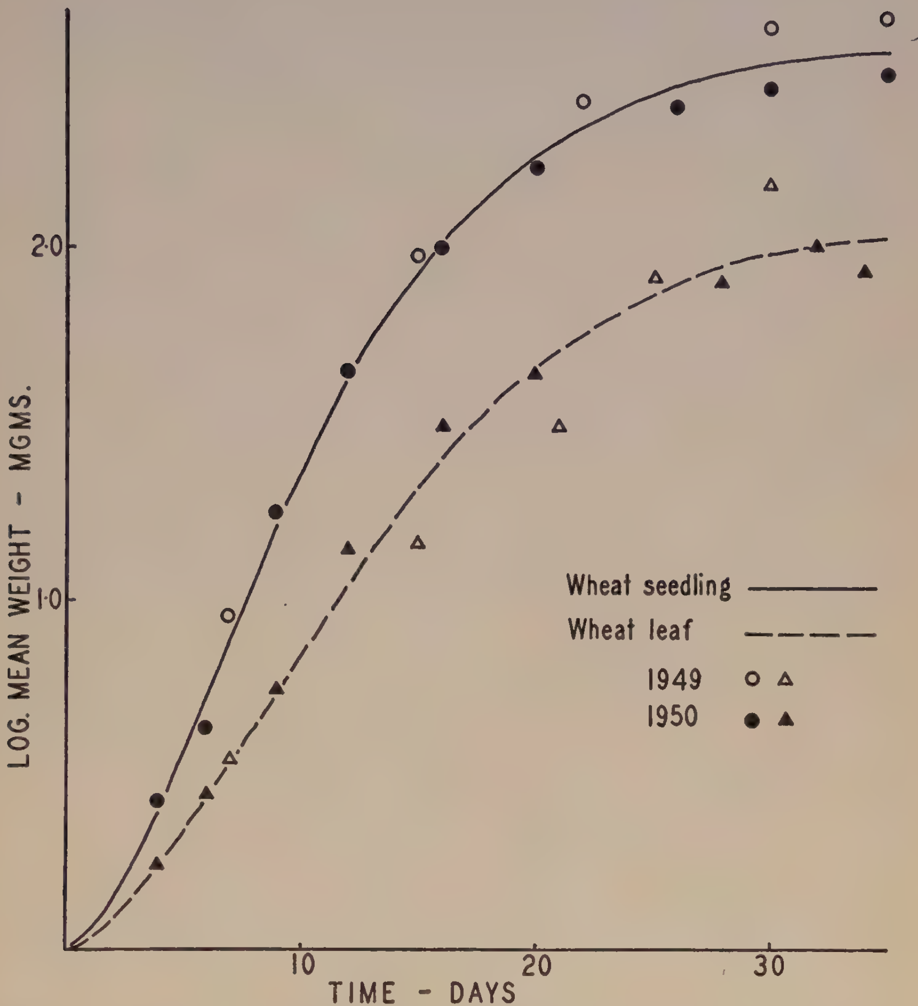

FIGURE 1. Growth of *A. orthogonia* larvae on wheat seedlings as compared with wheat leaf.

improvement in weights on seedlings as compared with leaves is similar at 50 per cent R.H. and at 70 per cent R.H., but is very much more marked in the plaster-bottomed tubes. The improved weights at 70 per cent R.H. are similar with seedlings and with leaf feeding, but in plaster-bottomed tubes there is again a markedly greater improvement on wheat seedlings as against that on cut leaf. At 70 per cent R.H. the food material remained moist longer than at 50 per cent, and in the plaster-bottomed tubes it remained moist longer still, but only with seedlings in plaster-bottomed tubes could the food continue to take up moisture through the root hairs while the larvae fed upon it. The very big difference between the weights at 21 days on seedlings in plaster-bottomed tubes as compared with seedlings at 70 per cent R.H. suggests that this higher water content is more

TABLE 2.—COMPARISON OF THE DURATION IN DAYS OF THE IMMATURE STAGES OF *Agrotis orthogonia* UNDER VARIOUS CONDITIONS

	Feeding stage		Prepupal resting stage		Pupal stage	
	Mean	Range	Mean	Range	Mean	Range
In the field	64	45-80	20	5-42	29.5	21-42
In the laboratory, 1949 and 1950—						
Fed wheat leaf (100 larvae, 29 adults)	49	38-63	15	11-20	24.0	20-28
Fed wheat seedlings (100 larvae, 44 adults)	36	28-47	10	8-16	23.0	20-26

important in the earlier stages. On a percentage of dry weight basis the nitrogen content of the leaf material would have been slightly higher than that of the seedling material (12).

No detailed observations were made on the adult stage. However, 311 fertile eggs were laid in a dish of dry soil passed through a 20-mesh screen, which was placed in a cage containing two females and three males reared on wheat seedling. A 25 per cent solution of sucrose was supplied as food for the adults. In the field up to 450 eggs may be laid by a single female (4, 9).

In Table 2, the duration of the immature stages on wheat leaf and wheat seedlings in plaster-bottomed tubes is compared with the duration in the field. The figures for the field are averages based on published observations (4, 9, 15); temperatures in the field of course would be lower in the early stages.

OTHER VARIATIONS IN FEEDING

Effects of Mineral Salts and 2,4-D

In 1951, four batches, each of 25 larvae, were reared in plaster-bottomed tubes on wheat seedlings in a manner already described, but the wheat seedlings on which they were fed were germinated with various treatments. First, in view of the many recent suggestions that insect damage to plants and development of insects on plants may be greatly affected by treatment of the plants with weed killers such as 2,4-D (5, 6, 10), two batches were fed on wheat germinated on filter paper moistened with water containing 5 p.p.m. acid equivalent of the sodium salt of 2,4-D. Considerable interest attaches to the effect of mineral deficiencies in plants on the insects which feed on them, and in consequence, to the possibility of insect control through fertilizer applications. On this account two batches were fed on wheat germinated by using a solution of nutrient salts containing the following salts (concentrations in gm. p. litre): KCl, 0.3; $MnSO_4 \cdot 7H_2O$, 0.45; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5; KH_2PO_4, 0.3; Ferric tartrate, 0.03; $MnCl_2$, 0.001; $ZnCl_2$, 0.0005; H_3BO_3, 0.0005; $CuCl_2$, 0.00001; (8, p. 63). One of these also included 2,4-D in the water. There were thus four batches, one germinated in plain distilled water, one each in 2,4-D solution and nutrient solution, and the fourth in a solution of both nutrients and 2,4-D.

TABLE 3.—WEIGHTS AND GROWTH RATES OF PALE WESTERN CUTWORM LARVAE FED ON WHEAT SEEDLINGS GERMINATED IN VARIOUS SOLUTIONS, 1951

	Distilled water	Water plus 5 p.p.m. 2,4-D	Water plus nutrient salts	Water plus 2,4-D plus nutrient salts
Mean wt. in mgm.	270.0	306.0	288.0	289.0
Mean duration of feeding period in days	40.5	40.5	43.0	41.0
Mean daily gain in weight in mgm.	6.7	7.6	6.7	7.0

The batches of larvae fed on each of these diets were distributed in a random manner in the two trays housing the plaster-bottomed tubes. The results obtained are summarized in Table 3. It was noticeable that fungus developed more rapidly on seedlings germinated in distilled water and in 2,4-D solution than on those germinated in nutrient solution. No pairs of treatments show significant differences ($P = 0.05$), either in the weights of the larvae they produced, or in the times that these took to mature. It would, therefore, seem unlikely that either the application of fertilizers or the use of 2,4-D as a weed killer would have any significant effect on the status of the pale western cutworm. The mortality was again high in these tests and it appears desirable to devise a means of keeping the humidity low while allowing access to water for the roots of the seedlings.

Observation of Cutting Off Activity

In order to observe the activity of larvae cutting off wheat plants, batches of six seeds were planted inside 6″-lengths of 2″-diameter glass tubing, one end of which was buried 1½″ into the soil in a pot. When the plants were well above ground, a layer of plaster of paris ¼″ thick was poured into the tube on top of the soil, and when this had set a young cutworm larva was introduced into each tube. Larvae were thus prevented from burrowing into the soil, and could readily be observed cutting off the plants. This usually took place at a height above the level of the plaster equivalent to about one-third of the length of the larva. After plants had been severed, larvae almost invariably continued to feed on the rooted stump of the plant, usually eating this down to the level of the plaster. They still apparently continued to feed at it, presumably on the fluids rising through the plant tissues. Larvae fed in this way grew rapidly, but none of them completed their development on the six plants with which they were confined.

DISCUSSION

It seems clear that the most satisfactory diet for the larvae of *Agrotis orthogonia* must contain more water than any ordinary cut plant leaf material. Without adequate water, total ingestion is so limited that nitrogen cannot be obtained fast enough for a normal growth rate. This importance of water in the diet is hardly surprising in view of the observations by Wigglesworth (16) on the permeability of the cuticle of soil-

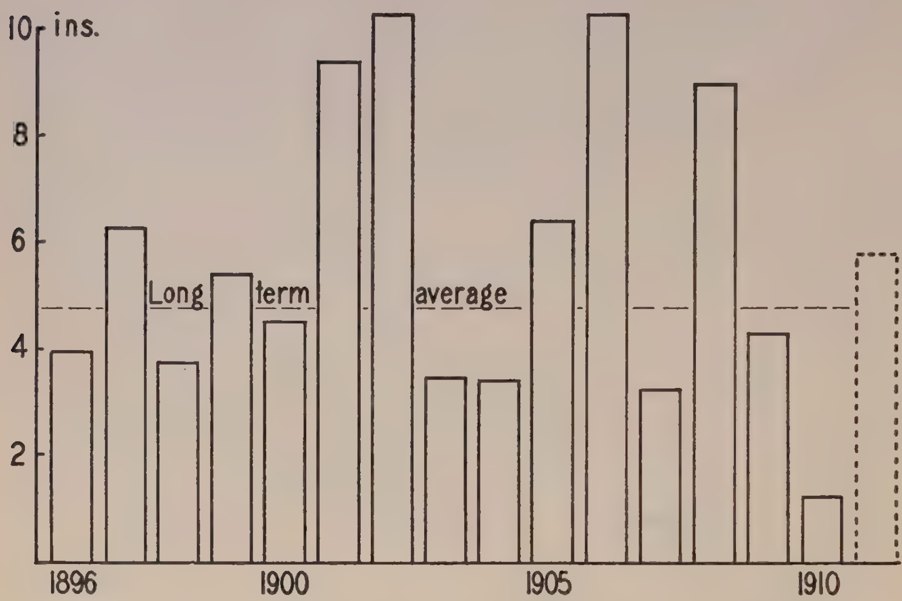

FIGURE 2. May and June rainfall in pale western cutworm area, 1896-1911.

dwelling insect larvae in general, and in view also of Cook's (2) observations on the meteorological conditions most favourable to this species, and its well known association with dry conditions. What is, perhaps, surprising is that this need for a high water content in the food remains manifest in individuals raised out of contact with the soil and under conditions of higher humidity. The very high rates of water loss which this insect has to face in nature dictate a plentiful source of replenishment. The habit of cutting through the stems of plants—and particularly of wheat—and utilizing the root systems of these to draw up water from depths to which it could never penetrate itself, is a clear adaptation to this need.

This dependence on high water content in the food, together with the apparent favourable effect of low humidities must have a bearing on the much discussed questions of the initial development of *Agrotis orthogonia* to pest status (1, 14, 15) and the causes of subsequent outbreaks (2, 3, 13, 14, 16). Agricultural development has been cited as the fundamental cause of the former, and few wet days during the larval feeding period of the latter. The Canada Year Book indicates considerable agricultural development within the range of this species for many years prior to 1911, but attention has not previously been drawn to the fact that 1910 was the first of these years when May and June were very dry. Figure 2 shows the averages of available figures for May and June rainfall in the area of abundance of this cutworm from 1896. In 1910 rainfall in these months was considerably less than half that in any previous year during this period; that in 1909 and 1910 together was 20 per cent less than that in any other two consecutive years.

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A PRELIMINARY STUDY ON THE EFFECT OF SOME FUNGICIDES ON THE ESTABLISHMENT OF FORAGE SEEDLINGS

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ABSTRACT

Several fungicides were used as seed treatments on eight grasses and five legumes commonly used in hays and pastures in Ontario. The seeds so treated were grown in different soil types that gave wide differences in seedling establishment. Legume seedlings were found to be more severely attacked by damping-off organisms than grass seedlings. Leytosan P gave good results when applied to most grasses and legumes, and excellent results when applied in excess amounts with red clover and alfalfa. Spraying the soil with the liquid fungicide Vancide 51 proved promising in establishing legumes.

Hay and pastures occupy a large acreage in Ontario, with the hay crop estimated at four million acres in 1951. The cost of establishing new seedlings of these crops each year means a considerable outlay on the part of the farmer. In some areas, failures to obtain a satisfactory stand are quite common. Any means by which a more desirable stand could be assured would be of great value.

Studies were started in the fall of 1949 to ascertain the effect of fungicides on the establishment of forage grasses and legumes. In all, four greenhouse tests have been run, each on different soils. The experiments conducted were approached from the agronomic standpoint and no effort was made to study the pathological organisms that attacked the seedlings.

Rose (6) reported an increase of 15 per cent in stand with sudan grass and soybeans when the seed had been treated with a fungicide. Brentzel (2) found treated seed to give more vigorous seedlings and improved stands. Kreitlow *et al.* (5) found that treating seed of sudan grass, red clover and alfalfa with fungicides protected the seedlings from damping-off in *Pythium* infested soil in the greenhouse. They found more damping-off occurring as the soil moisture was increased. In field tests, greatest response was obtained from treating sudan grass. Red clover responded more to seed treatment than alfalfa. Chilton and Graber (3) treated species of forage legumes and found N. I. Ceresan the best treatment. Highly significant increases were found in some species and no differences or some injury to others. Ulitos and Preston (7) report Phygon and Arasan at dosages of $\frac{1}{4}$ per cent, $\frac{1}{2}$ per cent and 1 per cent by weight of seed gave highly significant increases in germination when compared to untreated seed. Dow 9B and Spergon were also superior to untreated seed but less effective than the other two fungicides. Allison and Torrie (1) reported some fungicides to protect forage legumes against pre-emergence damping-off, but none was effective on post-emergence damping-off in compost infested soil. Under field conditions, they reported none of the fungicides used to be significantly beneficial with any of the legumes tested. Gerdemann (4) found that Arasan, Ceresan M and Phygon had no effect on red clover seed

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grown in wet or dry soil. With alfalfa, he found most seed treatments to be neither beneficial nor injurious in general. With sweet clover, Arasan increased emergence in the greenhouse but significantly reduced emergence in the field under drouth conditions.

MATERIALS AND METHODS

Several different species of forage crops were treated with different fungicides and grown in the greenhouse during the winters of 1949, 1950 and 1951. In all, eight fungicides were used. The fungicides were applied to the seed in three studies at the rate of $\frac{1}{2}$ per cent by weight of the seed. In another test three fungicides were applied at the rate of $\frac{1}{2}$ per cent, 1 per cent and in excess; while in still another experiment one fungicide was applied as a spray to the soil at different rates.

The seed was mixed with the fungicide in sealed containers and good coverage of the seed was obtained. Treatment was made three to four weeks before planting. An experimental unit consisted of 100 seeds. These units were planted in flats in the greenhouse at a uniform depth of half-inch. Randomized complete block design with four to six replications was used. The word "establishment" in this paper refers to the number of viable plants present 18 to 24 days after seeding when emergence was completed.

The soil used for the test in 1949 was a medium loam in which some damping-off had been noted previously. That used in the 1950 tests was a Burford loam collected from the college farm where good stands of grasses and legumes had usually been obtained. Two soils were used in 1951. One soil was a Vasey sand from Simcoe county, while the other, a Haldimand clay, was obtained from Haldimand county. In both these areas difficulty had been experienced in late years in obtaining satisfactory stands of new seedings of forage crops. In the Simcoe area, particular trouble has been experienced seeding down after a crop of potatoes. Two lots of soil were obtained from each of these areas, one where fair-to-good stands had been obtained, the other where poor catches of grasses and legumes were common. In the Simcoe area, these two lots of soil were

TABLE 1.—THE INITIAL GERMINATION OF THE FORAGE CROP SEEDS USED IN THE FUNGICIDE EXPERIMENTS IN PER CENT

Species	1949–50	1950–51	1951–52
Timothy	81	98	97
Brome grass	95	80	91
Orchard grass	87	86	96
Perennial rye	97	99	—
Meadow fescue	95	88	—
Red fescue	80	86	—
Reed canary	68	77	—
Kentucky blue	86	40	—
Alfalfa	86	70	91
Red clover	85	67	80
Alsike clover	98	95	—
White clover	77	73	—
Sweet clover	86	—	—

TABLE 2.—F AND C.V. VALUES FOR THE ANALYSES OF VARIANCE TO SUCCEEDING MEANS TABLES. ANALYSES RUN ON TRANSFORMED DATA

Species	Table 3		Table 4		Table 5								Table 6		Table 7				Table 8	
					Simcoe Soil				Haldimand Soil						Simcoe Poor		Haldimand Poor			
					Good		Poor		Good		Poor									
	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.	F	C.V.
Timothy	** 11.21	10.9	0.34	8.2	2.36	9.2	** 4.26	10.0	2.58	9.1	1.61	11.3								
Brome grass	** 12.80	6.1	** 5.18	8.2	* 2.81	4.0	1.09	8.8	2.37	3.8	* 2.86	6.3								
Orchard grass	** 11.32	7.9	* 2.60	7.7	** 6.33	5.9	* 3.31	7.0	0.45	4.4	1.79	5.2								
Perennial rye	* 3.37	5.6	0.95	6.8																
Meadow fescue	** 4.26	4.2	** 4.90	7.2																
Red fescue	2.17	5.2	** 5.13	6.8																
Reed canary	** 7.89	9.2	* 3.16	9.1																
Kentucky blue	* 2.95	6.2	2.29	16.5																
Alfalfa	** 5.62	13.3	** 9.33	15.6	* 3.28	27.6	1.54	42.4	* 2.84	10.4	0.28	19.8			* 12.66	21.3	4.21	7.3	** 5.87	21.0
Red clover	** 5.91	12.3	** 8.21	23.4	** 5.37	19.7	2.54	26.8	0.56	5.9	0.45	8.5	** 15.83	14.9	** 59.21	13.7	** 22.30	7.8	** 18.74	14.7
Alsike clover	** 5.86	9.5	0.82	8.9																
White clover	** 7.59	17.6	1.83	9.3																
Sweet clover	* 3.47	10.7																		

obtained from adjacent fields with the soil where a poor catch had been obtained being former potato-ground. The two lots from Haldimand county were from good and poor stand areas within the same field.

In this paper, soils used in the experiments where poor catches of forage seeds were found are designated as "poor", those from fair-to-good areas are designated as "good".

Table 1 gives the laboratory germination of the seed used in the experiments reported. The viability of Kentucky bluegrass seed used in 1950–1951 was low.

Fungicides used in the tests included Phygon, Spergon, Arasan, Semesan, Dow 9B, Ceresan M, Leytosan P and Vancide 51.

Before analysing the data, the determinations were transformed to angles according to the method outlined by Snedecor (7). An analysis of variance was made on these converted data and an appropriate L.S.D. figure computed. This figure was used to determine which comparisons among the converted means were significant. Differences among the converted means form the basis of the discussion reported in this paper on the effects of the fungicides on the establishment of grasses and legumes.

Table 2 shows the values of F of the analysis of variance carried out on the transformed data aud the coefficient of variability for each experiment. From this table it will be noted that the variability was relatively small in the experiments with grasses but generally larger in the tests with legumes.

Results 1949–1950

The results of the work carried on in the greenhouse during the winter of 1949–1950 are given in Table 3. In this test, all fungicides were applied at $\frac{1}{2}$ per cent by weight of the seed and four replications were used.

In this study timothy, brome and reed canary gave marked increases in establishment when treated with fungicides. With the other species the response from fungicides was not so great.

Phygon gave the highest establishment of any of the fungicides with timothy, giving twice the number of plants produced by the check. It also gave significant increases with brome, perennial rye, meadow fescue, reed canary, alfalfa and alsike. No adverse effect was found in any of the species.

Spergon increased the establishment with timothy, brome, reed canary and alsike only. These increases, though significant, were not great except in timothy, where the check was low. Although Spergon gave the highest number of plants in alsike, it reduced the number in the other four legumes, giving a significant reduction in red and white clover.

Arasan increased the establishment with timothy, meadow fescue and reed canary, but had little effect upon the other grass species. In the legumes, it increased alsike clover, sweet clover and alfalfa, but tended to decrease the number of plants of red clover.

Semesan increased the establishment in timothy, brome and reed canary. It gave good results with the legumes, giving increases with alfalfa, red clover, alsike and sweet clover, but having no effect upon the white clover.

TABLE 3.—THE EFFECT OF SOME FUNGICIDES UPON THE MEAN NUMBER OF VIABLE PLANTS PRODUCED PER 100 SEEDS PLANTED OF SOME FORAGE CROP SEEDS GROWN IN A LOAM SOIL DURING THE WINTER OF 1949–1950

Treatment	Timothy	Brome grass	Orchard grass	Perennial rye	Meadow fescue	Red fescue	Reed canary	Kentucky blue	Alfalfa	Red clover	Alsike clover	White clover	Sweet clover
Phygon	79.0	79.2	76.5	95.2	92.2	77.5	61.2	64.7	56.2	53.7	56.7	32.5	43.2
Spergon	55.0	77.5	66.2	91.0	85.2	76.7	47.0	68.7	25.7	29.7	59.0	8.2	33.7
Arasan	60.2	66.7	67.7	91.0	92.2	74.7	48.2	71.2	41.5	34.7	56.7	29.5	47.5
Semesan	63.0	84.5	73.5	89.2	89.7	73.0	46.0	77.5	49.0	55.5	55.2	30.0	52.7
Dow 9B	51.7	74.0	61.7	93.0	92.7	72.5	42.2	71.2	35.2	57.7	48.5	25.2	53.7
Ceresan M	69.5	87.5	90.0	94.7	92.0	82.2	50.2	67.2	36.5	60.0	36.7	40.2	50.7
Leytosan P	64.0	91.0	90.2	97.5	94.0	80.5	66.2	78.7	30.7	47.7	38.7	47.0	48.7
Check	27.5	63.7	66.7	87.2	86.0	75.5	35.5	71.2	32.2	42.2	46.2	27.2	37.5

TABLE 4.—THE EFFECT OF SOME FUNGICIDES UPON THE MEAN NUMBER OF VIABLE PLANTS PRODUCED PER 100 SEEDS PLANTED OF SOME FORAGE CROP SEEDS GROWN IN A CLAY-LOAM SOIL DURING THE WINTER 1950–1951

Treatments	Timothy	Brome grass	Orchard grass	Perennial rye	Meadow fescue	Red fescue	Reed canary	Kentucky blue	Alfalfa	Red clover	Alsike clover	White clover
Phygon	81.8	69.2	55.0	94.8	75.4	72.2	50.6	18.2	21.0	19.2	72.0	62.2
Spergon	78.6	54.4	49.2	93.2	77.4	79.6	39.8	24.6	8.2	10.4	71.6	58.4
Arasan	79.2	66.2	51.4	91.0	74.2	59.6	41.6	21.4	14.6	11.6	71.6	67.4
Semesan	79.4	64.2	55.2	89.4	84.6	69.8	38.4	21.2	7.8	13.0	67.8	53.0
Dow 9B	78.8	60.6	43.2	91.4	81.2	72.0	43.6	14.2	9.0	7.8	72.0	59.2
Ceresan M	84.2	65.8	55.0	95.4	88.8	72.8	47.6	25.8	10.2	31.4	62.8	57.6
Leytosan P	81.6	71.2	48.2	92.6	84.8	74.2	49.0	18.6	11.6	19.2	73.4	55.2
Check	79.6	49.6	47.6	92.0	70.6	64.0	36.8	15.2	6.8	6.8	69.2	53.8

Dow 9B increased establishment in timothy, brome, meadow fescue, red clover and sweet clover. With timothy, it proved to be one of the fungicides that gave the smallest number of plants, but with sweet clover, it gave the highest.

Ceresan M was effective with most species. It increased the establishment with all the grasses, except Kentucky blue and red fescue, gave the best establishment with red clover, and increased the number of plants with white and sweet clovers.

Leytosan P gave the best results of any of the fungicides tested. It gave the highest number of viable plants with six species and gave significant increases with two others.

Results in 1950–1951

Table 4 summarizes the results obtained with the same fungicides on a Burford loam soil. This soil, as previously mentioned, was obtained from the college farm where little trouble had been experienced in obtaining satisfactory stands. Under greenhouse conditions, however, damping-off was very marked, especially with red clover and alfalfa. The fungicides were applied to the various species at the rate of $\frac{1}{2}$ per cent by weight of the seed and five replications were used.

Brome again responded well to the fungicide treatments as did meadow fescue. Timothy, however, did not respond nor did perennial rye, Kentucky blue, white and alsike clovers.

Phygon gave significant increases in the establishment when compared with the check with brome, red fescue, reed canary, red clover and alfalfa. With alfalfa and reed canary, it gave the best stands of any of the treatments. Spergon increased the number of plants only with red fescue and with this species it was the most effective fungicide. Arasan gave increases only with brome and alfalfa, Semesan with brome, orchard, red clover and meadow fescue and Dow 9B with brome, red and meadow fescues.

Ceresan M and Leytosan P again gave good results. Ceresan M gave the highest establishment of any of the treatments with red clover and meadow fescue and also gave increases with brome, orchard, red fescue and reed canary. Leytosan P gave the best results with brome grass and also significant increases with red and meadow fescues, reed canary, alfalfa and red clover.

Results in 1951–1952

Table 5 gives the results of studies carried out during the winter of 1951–1952. Again in this study, the fungicides were applied at $\frac{1}{2}$ per cent by weight but six replications were used in each experiment.

With the species used in this study, the response to treatments was not as marked as in previous tests. Orchard and timothy, however, seemed to respond more to the treatments than brome, alfalfa and red clover.

Spargon in this test did not significantly increase or decrease the establishment with any species on any of the soils over that given by the check treatment. Arasan gave increases on some soils with timothy, brome, orchard and alfalfa. Dow 9B gave increases with orchard on the poor soil from Simcoe County and brome on the poor soil from Haldimand County. Vancide 51 gave increases with Orchard, and alfalfa on the Simcoe soils. Ceresan M gave significant increases with orchard, alfalfa and red clover.

TABLE 5.—THE EFFECT OF SOME FUNGICIDES UPON THE MEAN NUMBER OF VIABLE PLANTS PRODUCED PER HUNDRED SEEDS PLANTED OF SOME FORAGE CROP SEEDS GROWN DURING THE WINTER OF 1951–52. PLANTED IN SOIL OBTAINED FROM SIMCOE (SAND) AND HALDIMAND (CLAY) COUNTIES FROM FIELDS WHERE GOOD AND POOR STANDS OF NEW FORAGE SEEDLINGS WERE FOUND

Treatment	Timothy				Brome grass				Orchard grass				Alfalfa				Red clover			
	Simcoe soil		Haldimand soil		Simcoe soil		Haldimand soil		Simcoe soil		Haldimand soil		Simcoe soil		Haldimand soil		Simcoe soil		Haldimand soil	
	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor
Spergon	75.2	74.8	81.3	76.2	84.8	78.5	84.7	89.8	74.7	72.5	86.8	88.0	10.5	8.3	64.0	46.5	18.0	13.3	65.2	56.3
Arasan	78.2	88.3	83.2	82.5	87.7	78.3	90.0	93.5	82.7	81.7	88.0	88.3	24.3	10.7	71.8	46.0	27.2	22.7	68.5	57.2
Dow 9B	78.8	66.2	84.5	68.7	83.0	76.3	87.0	92.8	73.0	77.0	86.2	85.3	15.5	11.0	57.2	41.5	13.7	11.2	67.2	54.7
Vancide 51	81.5	80.2	72.8	82.3	86.0	80.0	88.0	89.8	85.2	78.2	85.3	88.3	24.7	13.5	70.5	49.2	28.8	21.2	65.0	55.7
Ceresan M	85.7	74.7	89.3	80.7	81.8	76.7	86.7	88.0	82.7	77.2	87.8	88.3	20.0	22.8	71.2	41.8	34.7	20.2	64.5	57.8
Check	71.5	76.2	85.8	75.3	82.5	69.7	86.0	86.5	74.7	68.2	87.7	82.7	11.5	10.5	59.3	46.8	20.3	11.5	65.0	52.5

TABLE 6.—THE EFFECT OF RATES OF APPLICATION OF THREE FUNGICIDES UPON THE MEAN NUMBER OF VIABLE PLANTS PRODUCED PER HUNDRED SEEDS PLANTED ON RED CLOVER GROWN DURING THE WINTER OF 1951–1952 ON SAND SOIL FROM SIMCOE COUNTY WHERE POOR CATCHES OF SEED WERE FOUND

Treatment rate	Viable plants per 100 seeds
Arasan .5%	19.2
Arasan 1%	22.0
Arasan excess	29.0
Ceresan M .5%	22.4
Ceresan M 1%	22.6
Ceresan M excess	23.0
Leytosan P .5%	37.8
Leytosan P 1%	42.4
Leytosan P excess	61.6
Check	16.2

Table 6 shows the effect of applying some fungicides at different rates of application on red clover. The fungicides used showed marked increases in establishment in previous tests, but since on the poor soil from Simcoe County they did not give good control of damping-off at the .5 per cent rate, heavier rates were used. The excess rate was where the fungicide was applied until free material was present. This free fungicide that did not adhere to the seed was screened off before counting and planting.

The excess rate of Arasan gave increases in establishment over the .5 per cent rate and also over the check. Rates of applying Ceresan M did not significantly affect the establishment. Leytosan P gave by far the best control of any fungicide in this study with the excess rate giving over twice the stand given by the check.

Due to the results obtained with Leytosan P in this test, another study was carried out on the poor soils from Haldimand and Simcoe counties with red clover and alfalfa. The results of this test using Leytosan P in excess are given in Table 7.

In three of the four tests shown, Leytosan P applied in excess significantly increased the establishment. Only with alfalfa on the poor soil from Haldimand was the increase not significant, though very marked.

TABLE 7.—THE EFFECT OF LEYTOSAN P IN EXCESS UPON THE MEAN NUMBER OF VIABLE PLANTS PRODUCED PER HUNDRED SEEDS PLANTED OF RED CLOVER AND ALFALFA GROWN DURING THE WINTER OF 1951–1952 ON SOIL FROM HALDIMAND AND SIMCOE COUNTIES WHERE POOR CATCHES OF SEED HAD BEEN OBTAINED

Treatment	Red clover		Alfalfa	
	Simcoe poor	Haldimand poor	Simcoe poor	Haldimand poor
Leytosan P excess	63.5	64.8	66.3	64.8
Check	23.7	47.3	34.5	47.5

TABLE 8.—THE EFFECT OF APPLYING DIFFERENT RATES OF VANCIDE 51 AS A SPRAY TO THE SOIL UPON THE MEAN NUMBERS OF VIABLE PLANTS PRODUCED PER HUNDRED SEEDS PLANTED OF RED CLOVER AND ALFALFA. GROWN DURING THE WINTER OF 1951–52 ON SAND SOIL FROM SIMCOE COUNTY WHERE POOR CATCHES OF SEED WERE FOUND

Rate of application	Red clover	Alfalfa
1-lb. per 1000 sq. ft.	59.0	53.2
$\frac{3}{4}$-lb. " " " "	56.5	60.0
$\frac{1}{2}$-lb. " " " "	49.5	60.7
$\frac{1}{4}$-lb. " " " "	56.2	53.2
$\frac{1}{8}$-lb. " " " "	28.0	31.5
$\frac{1}{16}$-lb. " " " "	32.7	32.5
Check	8.2	17.7

Vancide 51, a liquid fungicide, was tried as a spray, applied to the surface of the soil to control the damping-off organisms on the poor soil from Simcoe County. It was thought that this might have a practical value in the field if an economical quantity could be used. The results of this test on alfalfa and red clover are given in Table 8.

In this test, the variability was high with both species. However, as a preliminary study, it does show some promise. There appears to be a break between the $\frac{1}{4}$ and $\frac{1}{8}$-lb. rates. The $\frac{1}{4}$-lb. rate would mean about 11 lb. per acre, which at present might be expensive. If the cost permitted and good control could be assured, spraying such a fungicide with a sprayer at planting-time might prove to be beneficial.

DISCUSSION

Post-emergence damping-off was prevalent in the tests on all soils. Some grass seedlings seemed to die but the legumes were most seriously affected. The legume seedlings seemed to damp-off after they had emerged and usually before the first true leaves were well developed. Once this stage of growth had passed the seedlings were less affected.

In the greenhouse studies carried out, the damping-off organisms seemed to attack portions of some rows rather than a uniform attack along the row. This caused variations between replications and resulted in relatively high coefficients of variability in some cases. In later experiments more replications were used but the variability remained high with some of the legume tests.

In the 1949–1950 studies, most of the grasses and legumes responded to some fungicides. The increases obtained with the different species varied from slight to marked. Similar results were obtained in 1950–1951 but here several species did not respond to treatment. Alsike and white clover did not damp-off nearly as much on this soil as on the one used in the previous year. Alfalfa and red clover, however, were severely attacked. Timothy gave marked increases in 1949–1950 but no increases in 1950–1951. Brome, however, responded to treatment both years. Since different seed lots and soils were used each year, perhaps the species comparisons should not be considered too critical.

From the results obtained in the first two studies it would appear that different soils might contain organisms varying in amounts and pathogenicity. This was further exemplified in the studies of 1951–1952. Here the establishment obtained on the Haldimand soil was much higher, in every case, than that obtained on the Simcoe soil, the difference in establishment being especially noticeable with red clover and alfalfa. In most cases the good soil gave a higher establishment than the poor soil from the same vicinity. In most cases the seedlings did not establish as well on the good soil from the Simcoe area as on the poor soil from the Haldimand area.

From the results with Leytosan P and Ceresan M in these experiments, it would appear that they hold special promise. Leytosan P when applied in excess amounts gave excellent establishment with red clover and alfalfa. With these two legumes this fungicide gave equally good establishment on the Haldimand and Simcoe soils though wide differences appeared between the checks. Though an increase was obtained in this test, caution must be taken when applying fungicides at heavy rates since in a similar study with brome grass the excess amount of Leytosan P was so severe that no seedlings established.

Vancide 51 applied as a spray to the surface of the soil gave interesting results. There is a possibility that this fungicide used as a spray to the soil and coupled with a satisfactory seed treatment might prove to be valuable in greenhouse experimentation at least.

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THE CLIMATE OF NORTHERN ONTARIO

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ABSTRACT

Northern Ontario, lying between the upper Great Lakes and Hudson Bay, has a modified continental climate. Since the longest weather records in the province show only a small rise in average temperatures during the last century the present normals may be expected to approximate the climate of the future. In summer (July) the settled sections have temperatures of 61° to 67° F. In January the isotherm of 0° F. runs through the northern clay belts. In the spring the curve of average temperatures reaches 42° F. about April 24 in the warmest sections and May 5 in the Cochrane-Kapuskasing district, but the average date of the last killing frost is a month or more later. In the fall, the northern settlements cannot expect frost-free weather after the first week in September while a comparable date at North Bay, Sudbury, and Fort Frances is September 20. The average precipitation varies from 20.5 to 42.6 in. and fortunately it is heavier in summer than in winter. Serious deficiencies of soil moisture are not frequent. The surplus water is about 6 in. less on the Manitoba than on the Quebec boundary. The area is divided into 8 climatic regions, the characteristics of which are listed in a table.

The present paper presents an account of the climate of northern Ontario which is complementary to a similar account of the climate of southern Ontario published some years ago (4).

The purpose of the analysis is to define those characteristics of the climate that have a bearing on the flora and fauna of the region and on the nature of its soils and their cultivation. These same conditions of temperature, rainfall, snowfall, wind and sunshine have implications also for human living conditions and the construction and maintenance of buildings and roads. The description of climatic factors is based on the records compiled by the Meteorological Service of Canada from the observations of about forty-five stations with long-term records. These stations for the most part are operated by public-spirited voluntary observers.

At present, northern Ontario produces less than 5 per cent of the agricultural products of the province. The climate is marginal for many valuable crops and presents difficulties to the farmer in the handling of the soil, crops and live stock. However, the region includes one of the larger unsettled areas of potential farmland in Canada, so it is particularly important to have a description of the climate, as a guide to settlers. Farming in the Northern Clay Belt is a difficult undertaking and the settler may well seek a location having as few handicaps as possible.

Northern Ontario is a large area covering about 313,000 square miles (200 million acres). North Bay is just north of latitude 46° N., while the northernmost point of the province on Hudson Bay is near latitude 57° N. In other words, the area extends more than 700 miles from south to north, and about the same distance from east to west. Most of it is uninhabited, settlement being practically all south of latitude 50°, 30′ N.The only weather station north of Moose Factory near 51° 30′ N. is at Trout Lake, close to latitude 54° N. and it has only an 8-year record. Some valuable information for the northern part has been borrowed from Port Nelson, Churchill, and Norway House in Manitoba.

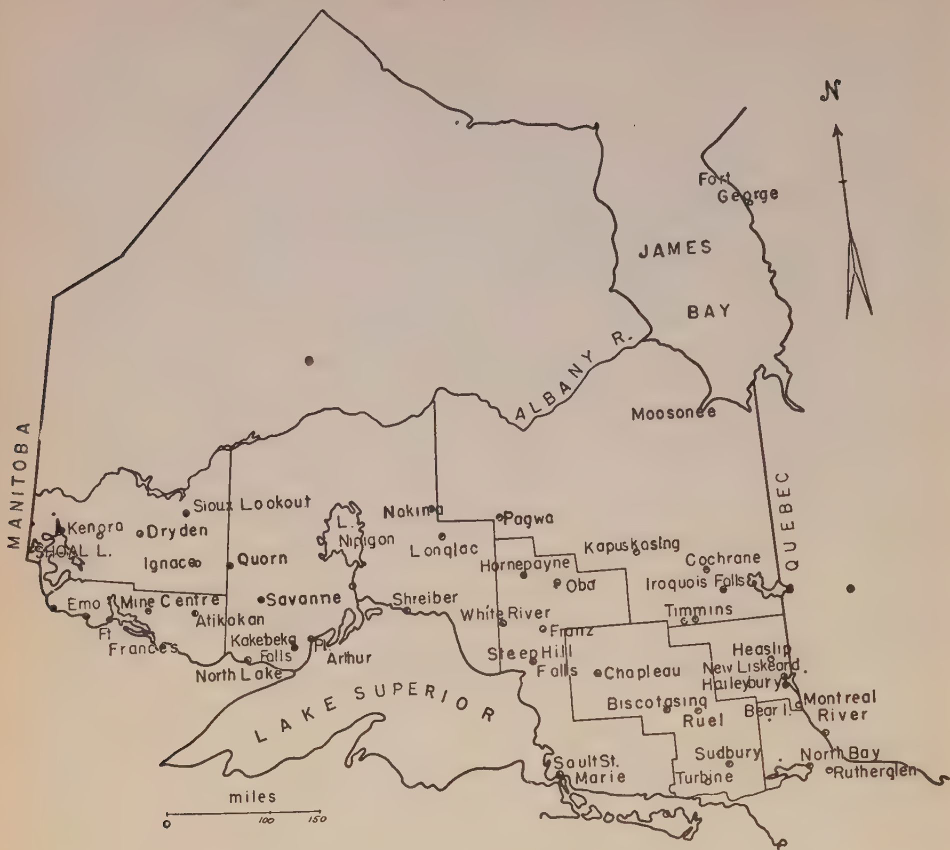


FIGURE 1. Weather stations.

The area is one of low relief, most of it less than 1000 feet above sea level. Those areas above 1500′ A.T. are shown on Figure 2, as are the few ridges of rock which rise over 2000 feet above sea level. The height of land between the Great Lakes-St. Lawrence drainage and the Hudson Bay drainage lies in a wide crescent north of Lake Superior extending westward to Lake of the Woods and eastward to Kirkland Lake. Although it is only a few hundred feet high it exerts an appreciable effect on climate which will be seen on both temperature and precipitation maps.

A broad, boggy lowland around James Bay is underlain by stratified limestones of Palaeozoic age, as is a small area around Lake Temiskaming. The remainder of the area is part of the Canadian Shield and consists of knobs and low ridges of Precambian rocks on which there is usually a thin discontinuous mantle of sandy drift. However, in several large areas there are deep beds of clay, silt and sand in the depressions and these underlie the main farming sections.

The sediments of glacial Lake Agassiz provide the deeper soils of the Rainy River-Fort Frances district. In the Thunder Bay district there are the fine sands and clays of Lake Algonquin. By far the largest and most continuous of these clay plains is the Northern Clay Belt, stretching 125 miles from Hearst to Cochrane in a belt up to 40 miles wide. This belt

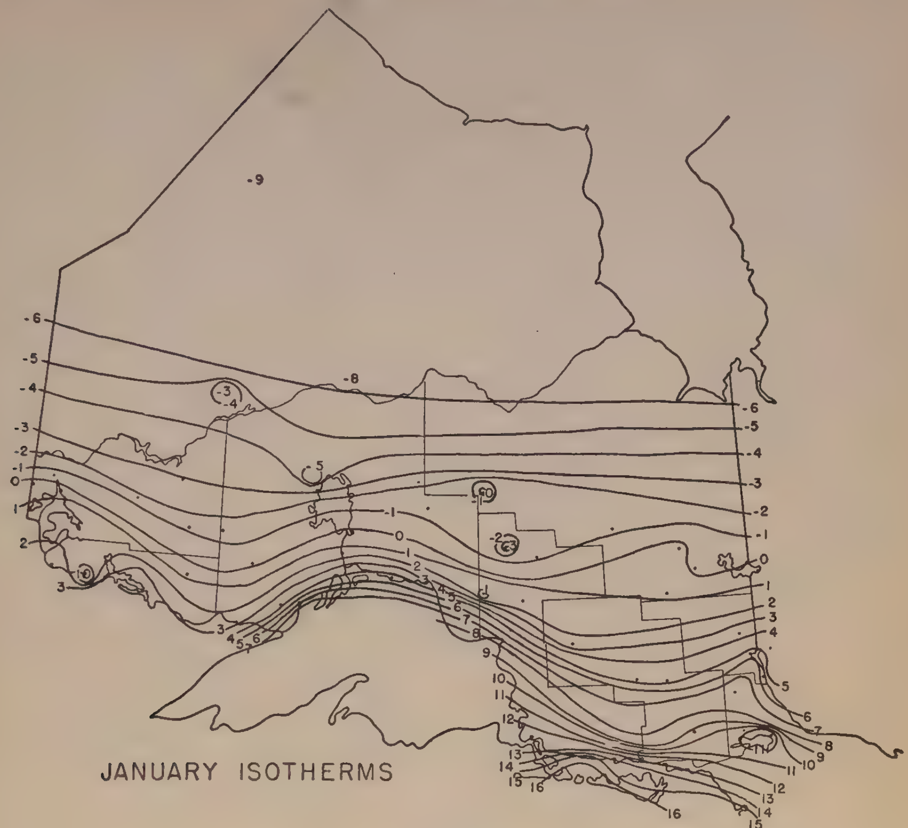


FIGURE 3

extends into Quebec east of Lake Abitibi and discontinuous clay beds occur west of Hearst. Its elevation is generally between 700′ and 900′ above sea level. This clay plain is crossed from north to south by a series of sand ridges, and these dry, elevated sites are favoured for farming while the peat-bogs in the depressions are less suitable for agricultural development. The Temiskaming Clay Belt is a fairly uniform area of clay now almost all cleared and cultivated. Between North Bay and Sault Ste. Marie the settlements have developed on the clays and sands.

On the north, Hudson Bay and James Bay exert a cooling effect on Northern Ontario and they are aided by extensive tracts of water-covered, floating bog on the bordering lowland. The upper Great Lakes serve to ameliorate temperatures in the southern part of the area.

TEMPERATURE

It is commonly believed that the climate of the area was formerly colder than at present. It is, therefore, desirable to examine the records for any evidence of amelioration of climate or of the existence in the past of conditions unlikely to recur in the future.

An examination of the records for White River, which cover a period of 60 years, shows a temperature-rise of a little over 1.0° F. during the last 30 years, but this followed a decline of over 0.5° F. in the 30 years preceding.

FIGURE 4

A rise of one-half a degree in 60 years is scarcely significant. During the same period the temperatures of the Toronto Station show a rise of about 1.5 degrees. No doubt some of this must be attributed to the expansion of the city and the pall of smoky air above it. These increases are of a small order and do not compare with those of about 3° F. recorded in Iceland, for instance, where the rapidly receding glaciers are also pointed to as evidence of a warming climate (3). Thus, on the basis of these long records, it is safe to assume that temperatures in northern Ontario in the future will not differ greatly from those now recorded.

Normal Monthly Temperatures

As a first step in the analysis of temperatures, isothermal maps (Figures 3–14) are given for each month. These are based on normals calculated from records up to the end of 1947. July temperatures represent midsummer conditions. A broad belt north and northeast of Lake Superior, including the Northern Clay Belt, has uniform mean temperatures of 61 to 63 degrees. From this belt the temperature increases towards Lake of the Woods on one hand and Lake Nipissing on the other, reaching a maximum of 67 degrees. The vicinities of Kenora, Fort Frances, Sudbury and North Bay have slightly warmer midsummers than the upland in Bruce, Grey, Dufferin and northern Wellington counties in southern Ontario.

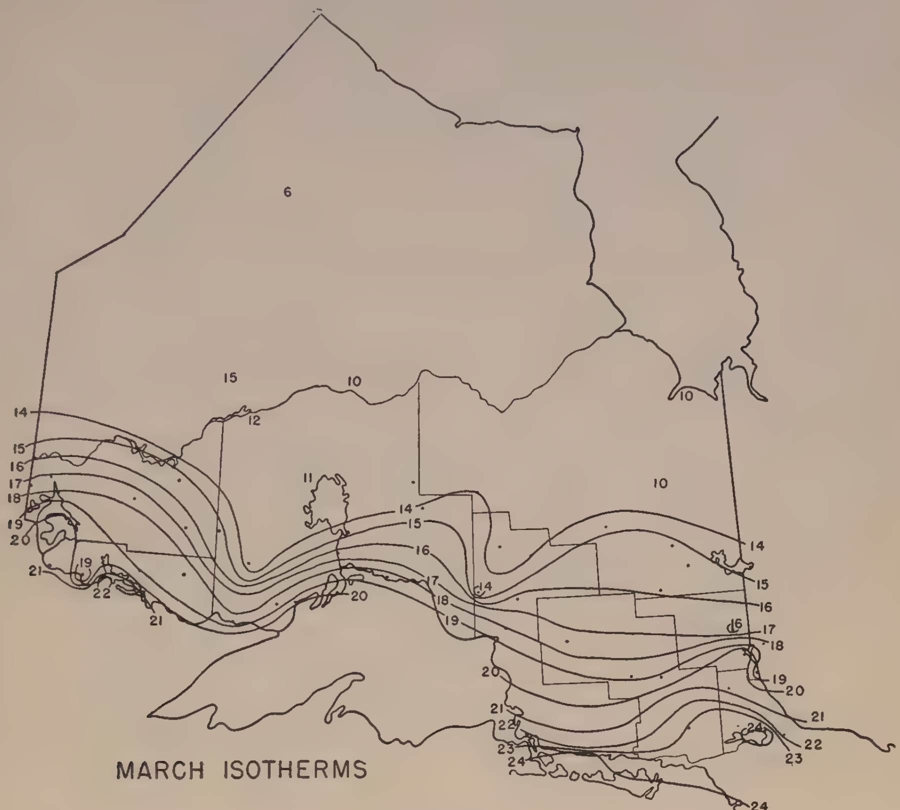


FIGURE 5

Taking the January isotherms as a measure of midwinter temperature it may be seen that the border along the north shore of Lake Huron and around Lake Nipissing has a normal temperature of 12 degrees and Sault Ste. Marie records 14 degrees. The zero degree line stretches from Lake Abitibi to Lake of the Woods, passing south of Lake Nipigon. The northernmost tip of the province is not far from Port Nelson, Manitoba, where the January normal is 17° below zero. Dryden, with a January normal of 3° below zero, is the coldest of the farming areas; the Northern Clay Belt is not much warmer while both Thunder Bay and the Temiskaming Clay Belt lie between the 3° and 6° isotherms. In comparison, the warmest sections of southern Ontario have mean January temperatures of 25 degrees.

The December, February and March isothermal maps may be examined for further detail about winter conditions. The warming effect of Lake Superior is quite marked in winter but in this sparsely settled area relatively few people are affected, apart from the more populated vicinities of Fort William-Port Arthur and Sault Ste. Marie. The east-west trend of the isotherms west of Thunder Bay should be noted as it is in contrast to their trend in summer. Temperature differences are greater in winter than in summer; for instance the difference between North Bay and Moosonee is 15 degrees in February and 6 in July.

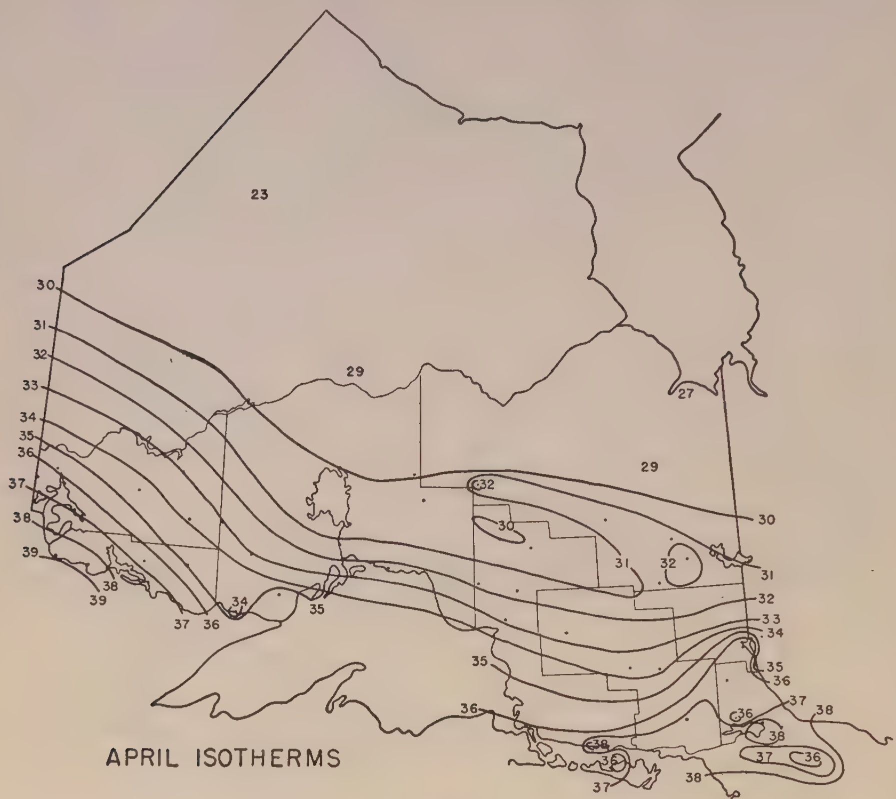


FIGURE 6

The more continental climate west of Lake Superior—one feature of which is a rapid warming in the spring— is indicated by the April isotherms which swing northwestward from Thunder Bay to the Manitoba border. The temperature at Fort Frances is similar to that of Sault Ste. Marie at this time of year although much colder in winter. At Kapuskasing the mean temperature in the middle of April (31°) is still below freezing. For comparison the Windsor and Leamington mean temperature is 46 degrees and at Ottawa it is 41 degrees.

May and June are important with respect to spring planting and the early part of the growing season. On May 1 at Kapuskasing the highest daytime temperatures average 51° but the lowest night temperatures average 29 degrees, still below freezing. However, a week later the average daily minimum temperature is above 32° and the mean temperature has risen above 42° which is sometimes used as a value to mark the beginning of crop-growth. The average date of the first work on the land at the Kapuskasing Experiment Station is May 11 while throughout the Northern Clay Belt the average date of first seeding is about May 20. The more rapid warming up of the Rainy River districts as compared to the Thunder Bay, Sault Ste. Marie and Kapuskasing-Cochrane districts is shown by the May and June temperature maps. The temperatures of the New Liskeard section almost keep pace with those of Rainy River during these months.

FIGURE 7

August and September isotherms follow much the same pattern as that shown by the July map. The rocky wooded country on the height of land southwest and east of Lake Nipigon is a cool belt; the Northern Clay belt is two or three degrees warmer, although the situation of its weather stations in open country may be responsible for the difference recorded. The warmer of the settled sections are the Rainy River, Temiskaming Clay Belt and North Bay-Sudbury areas.

With the approach of winter, in October and November, the isotherms assume more nearly a straight east-west trend. The Rainy River and North Bay areas have lost much of their summer temperature advantage by November. Freezing temperatures occur regularly in these areas after the middle of October, while towards the end of November the highest temperatures on most days fail to rise above the freezing point.

Extremes of Temperature

The highest and the lowest temperatures ever recorded at each station are interesting extremes. The figures are not strictly comparable since the various stations have not operated during the same period of years. However, the majority of the temperature extremes, both low and high, have occurred within the last 20 years. The lows at most stations occurred in 1933, 1934 or 1935 while the highs mostly occurred on July 11, 12 or 13, 1936.

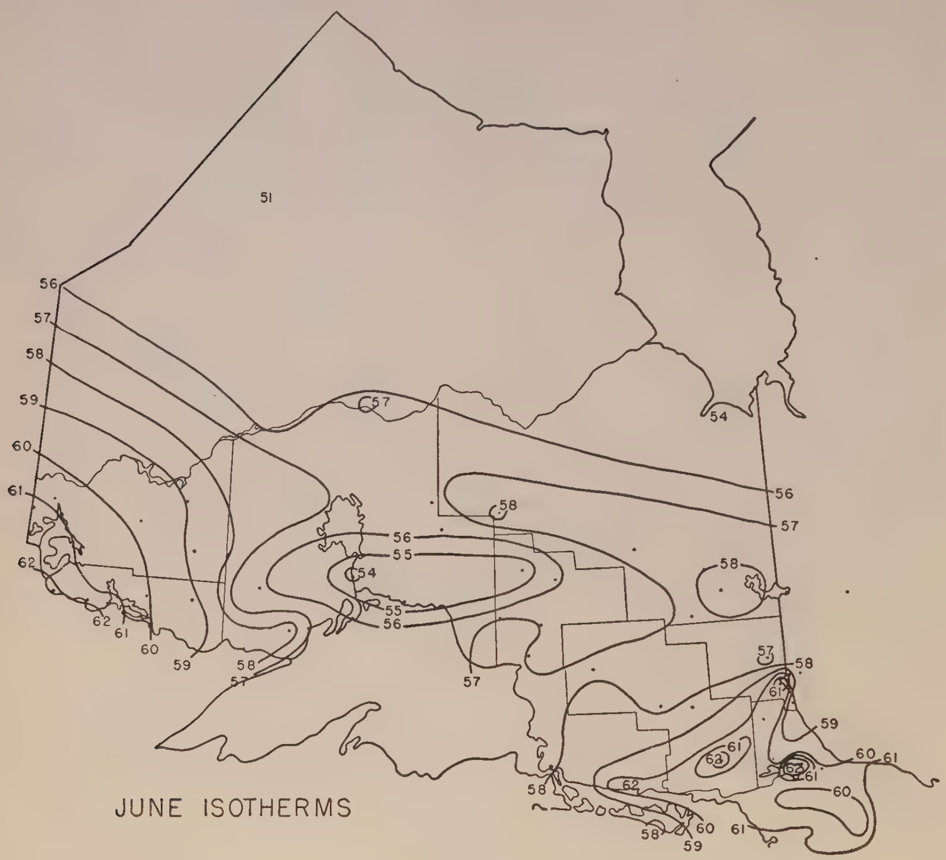


FIGURE 8

The lowest temperature ever officially recorded in Ontario is 73° below zero. It was registered at Iroquois Falls on a clear, still night on January 23, 1935. On the same night the White River station registered −61° and Hornepayne 63° below zero. Most of the lows are between 50° and 60° below zero. The highest temperature ever recorded at each station is usually over 100° and three stations, Fort Frances, Atikokan and Biscotasing, registered 108°. Figure 15 is a map of the low temperature extremes.

On the average the highest temperatures reached in summer are between 89° and 93° while the lowest temperatures of winter are from 30° to 50° below zero. White River with −51° has the lowest average winter extreme of any Ontario Station, while Chapleau with −47° is next in line.

Daily Range of Temperature

The daily range of temperature, or the difference between the temperature registered by the maximum and minimum thermometers each day, varies considerably. Figure 16 shows the daily range in July. Generally it is around 28° on the rocky uplands, grading down to 20° on the shores of the Great Lakes and other good-sized lakes such as Lake of the Woods, Lake Nipigon and Lake Temiskaming. No doubt these figures are affected by the terrain surrounding the weather stations.

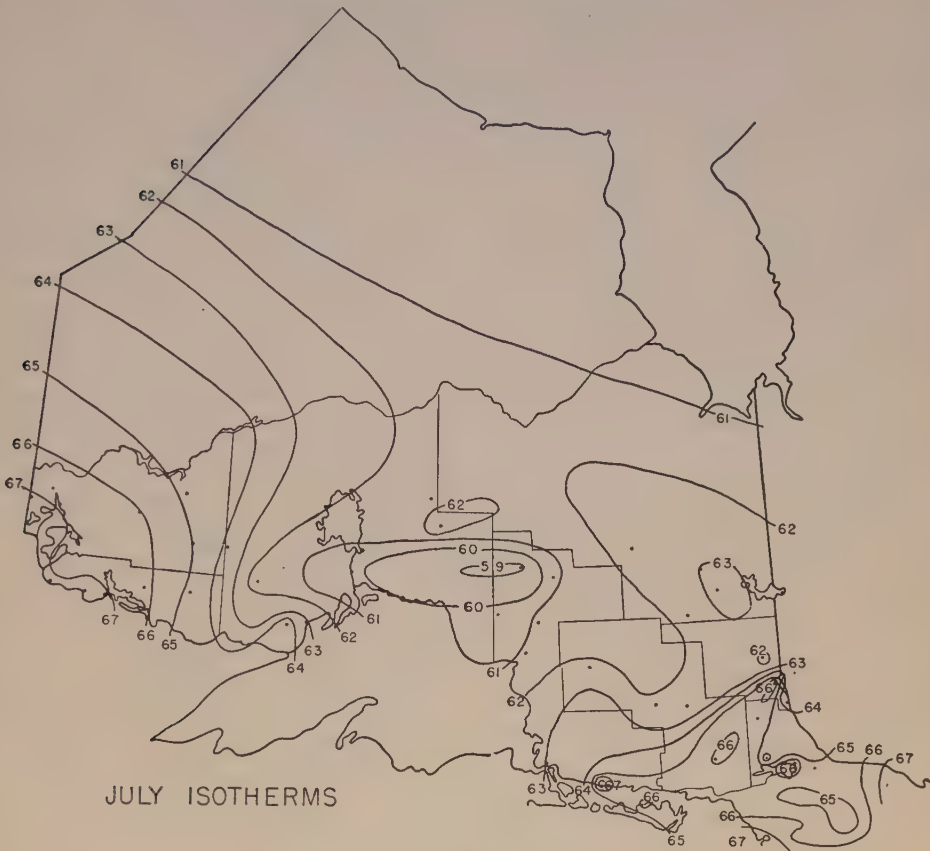


FIGURE 9

The Growing Season

It is quite common in descriptions of climate to define a 'growing period' as the period when the mean temperature is 42° and over. This criterion proved useful in southern Ontario, especially in comparing the various sections. The date when the curve of mean temperature rises above 42° is called the 'beginning', and the date when it falls below the 42° level in the autumn is the 'end' of the growing season. These dates supplement the information about the incidence of the last frosts in the spring and the first in the autumn.

On this basis the growing season begins about April 24 in the Rainy River and North Bay-Sudbury regions. The Temiskaming Clay Belt and Thunder Bay are a week later, while May 5 is the comparable date for the Northern Clay Belt. In the fall the growing season ends in the Clay Belt about October 8 while in the Temiskaming Clay Belt, North Bay and Sudbury areas and Rainy River settlement the period with normal temperatures above 42° ends on October 15 to 17. The two sets of dates for spring and autumn and the length of the period between them are shown in the maps called Figures 17, 18 and 19. They may be compared with similar maps for southern Ontario.

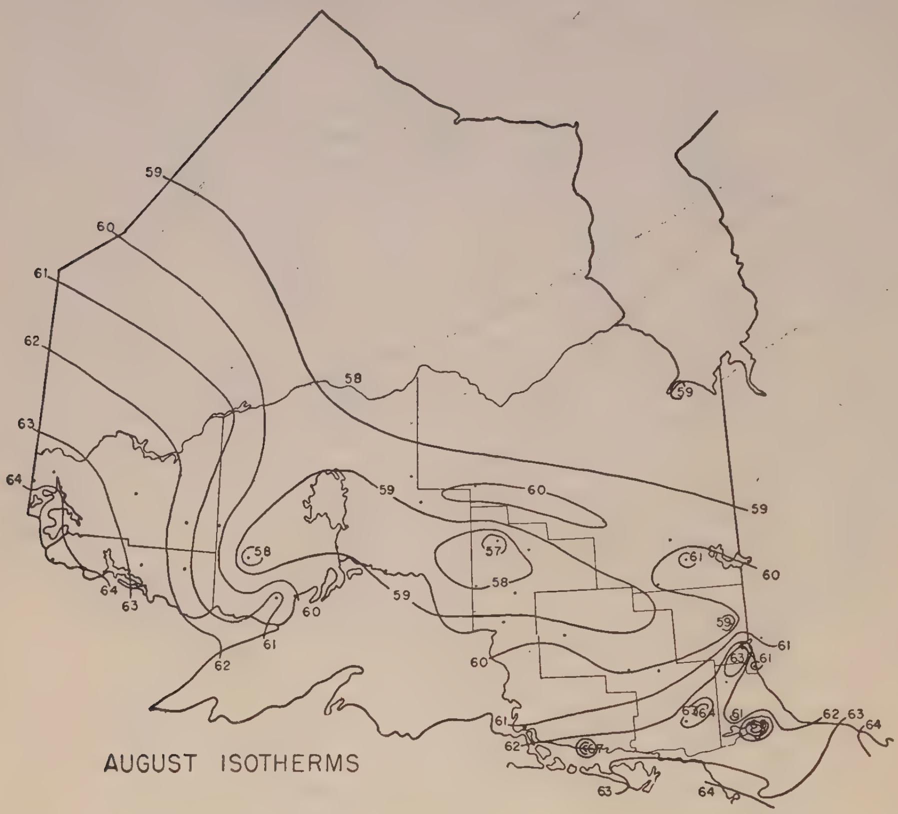


FIGURE 10

Frosts

The period free of killing frosts is in some respect more important than the growing period based on 42°. The dates of the last spring frosts and the first fall frosts are of special interest in Northern Ontario where the summers are so short.

It is common knowledge that considerable local variation occurs in the incidence of frosts. The lay of the land, the presence of lakes, the type of soil and the size of the clearings all have an effect. Low ground is notoriously frosty particularly in Northern Ontario where most depressions are peat-bogs. Even when the peat is on higher ground the crops growing on it are much more susceptible to frost than those on adjacent clay soil at the same elevation. Small clearings are considered to be frost traps, but the exact effect of opening up a broad tract of land for farms is hard to appraise. It is to be expected, then, that neighbouring weather stations will show variations in frost dates; in fact it is surprising that they are not more irregular.

Most descriptions of climate give the average dates of the last spring and first fall frosts. In approximately 50 per cent of the years, killing frosts have occurred after and before these respective dates. Two maps, Figures 20 and 21, show median dates; that is the dates after and before which spring and fall frosts occurred in exactly 50 per cent of the years.

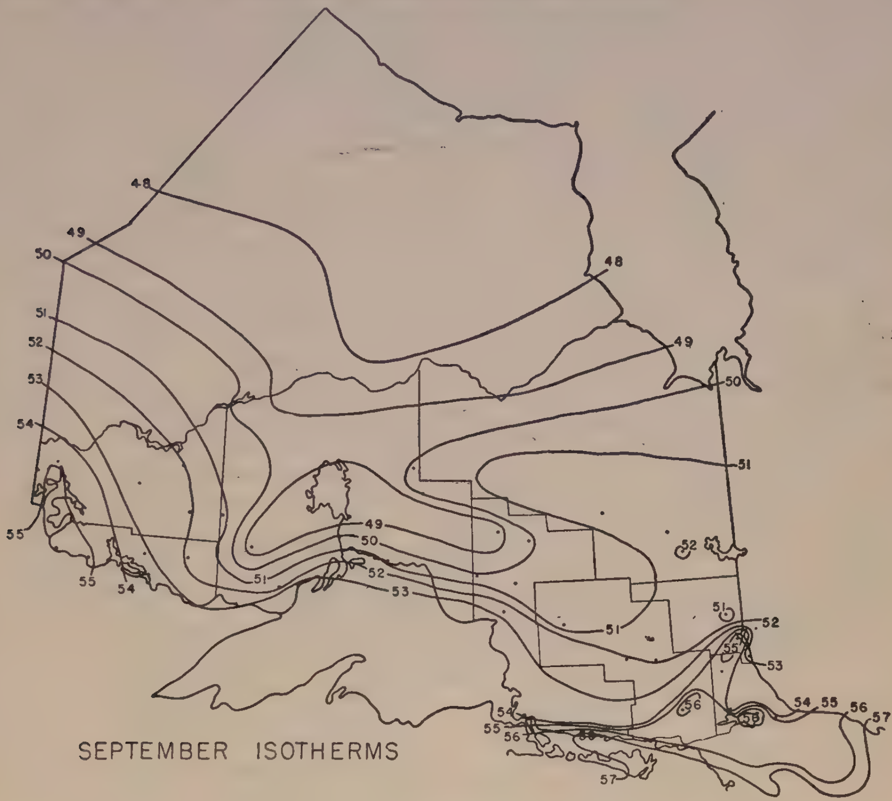


FIGURE 11

A third map gives the length of the periods between them. These median, or 50 per cent dates, are about the same as the average dates, and are about a week earlier in the spring and later in the fall than dates which allow a 75 per cent chance of escaping killing frosts.

These maps show a frosty belt over the height of land. No doubt the local relief affects the temperatures recorded at the various stations. Most of the stations are in valleys and Savanne and Hornepayne are the most susceptible to frosts, the dates of their last spring and first fall frosts being July 11 and 10 and July 22 and 25 respectively. The dividing-line between spring and fall is arbitrarily placed on July 15. There is practically no frost-free period at these stations, only 12 days in three out of four years. In the Northern Clay Belt the period between June 20 and August 29 is frost-free except in one year in four, when it is shorter. The most favoured section is the north shore of Lake Huron and Georgian Bay where in 50 per cent of the years there is no frost between the last week of May and the tenth of September.

The dates of June 15 and September 1 are commonly used in connection with crop growth in Northern Ontario. The per cent of years having frosts after the middle of June and before the first of September are shown on the following map, Figure 23. In each case the percentages vary from zero in the North Bay-Sudbury-Sault Ste. Marie area to 100, or nearly, at certain stations on the Shield.

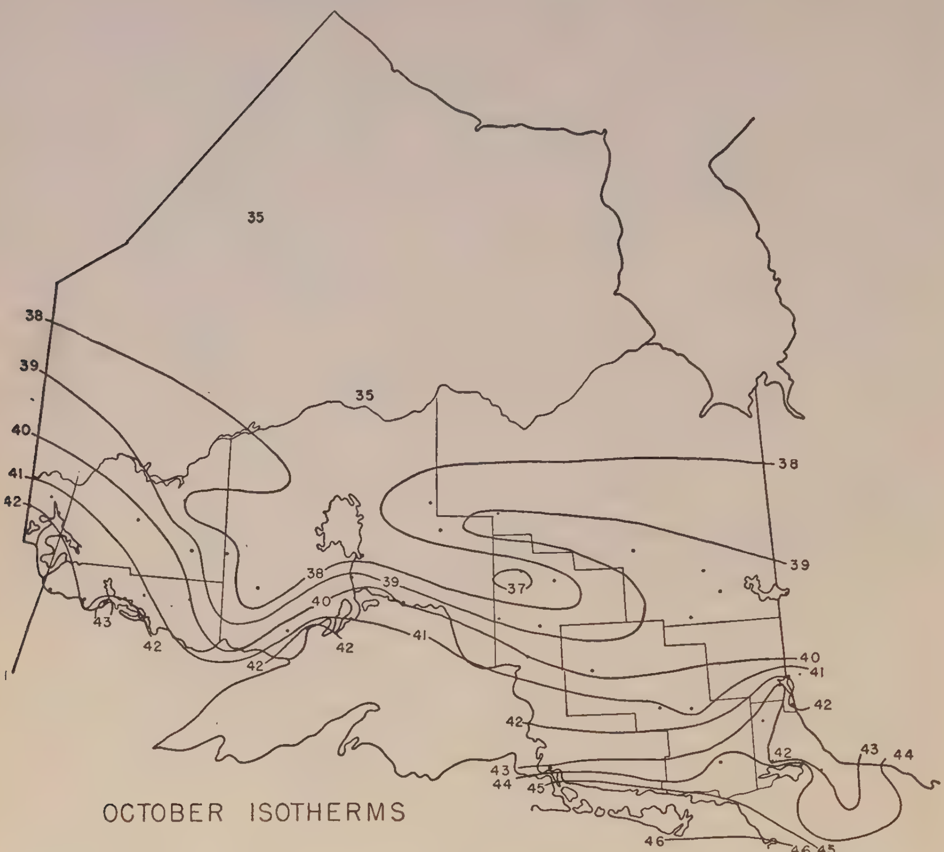


FIGURE 12

Effective Heat

In describing the climate of southern Ontario use was made of the Thornthwaite temperature efficiency (T–E) index (7). More recently Thornthwaite has devised another method of analysing climate which includes a new temperature index (9). It is actually an estimate of the amount of water that would evaporate from the soil and transpire from the plant cover if there were adequate moisture continuously in the soil. It is used to assess the adequacy of rainfall but also serves as a heat index. Figure 24 is a map of potential evapotranspiration; it will be considered now as a heat index and later as a value for optimum moisture requirement.

This potential evapotranspiration map bears some resemblance to July and August isotherms but it accentuates certain variations. The Rainy River region is clearly set apart from the Thunder Bay area, being similar to the belt north of Georgian Bay and around North Bay and New Liskeard. The Northern Clay Belt receives the least heat of any of the settled areas, the values of 19 inches at Kapuskasing and Cochrane being nearly 3 inches lower than at Sudbury and nearly 7 inches lower than at Leamington.

FIGURE 13

PRECIPITATION

Annual Precipitation

Precipitation includes both rain and snow with the latter recalculated to its rainfall equivalent in inches according to a ratio of ten to one. Figure 25 is a map of isohyets for northern Ontario.

The highest precipitation in the province (42.6 in.) is recorded at Helen Mine (Steep Hill Falls) and no other station in northern Ontario even approaches it. Rainfall and snowfall are both high. There are no other stations on the western slopes of the rocky ridges east of Lake Superior but Helen Mine probably represents that zone fairly well. Crystal Falls near the town of Sturgeon Falls records about 35 inches, which is about 5 inches higher than the North Bay and Sudbury normals. In the few years of its operation North Bay airport north of the city on higher ground has recorded more precipitation than the old North Bay station. The Lake Temiskaming area receives just over 30 inches. Iroquois Falls and Wawaitin Falls near Timmins show 32 and 31 inches respectively, but 26 inches and 28 inches at Cochrane and Kapuskasing more fairly represent the Clay Belt. At the Lakehead, Port Arthur receives an average of only 24 inches of precipitation and Emo a similar amount. Kenora, Dryden, Sioux

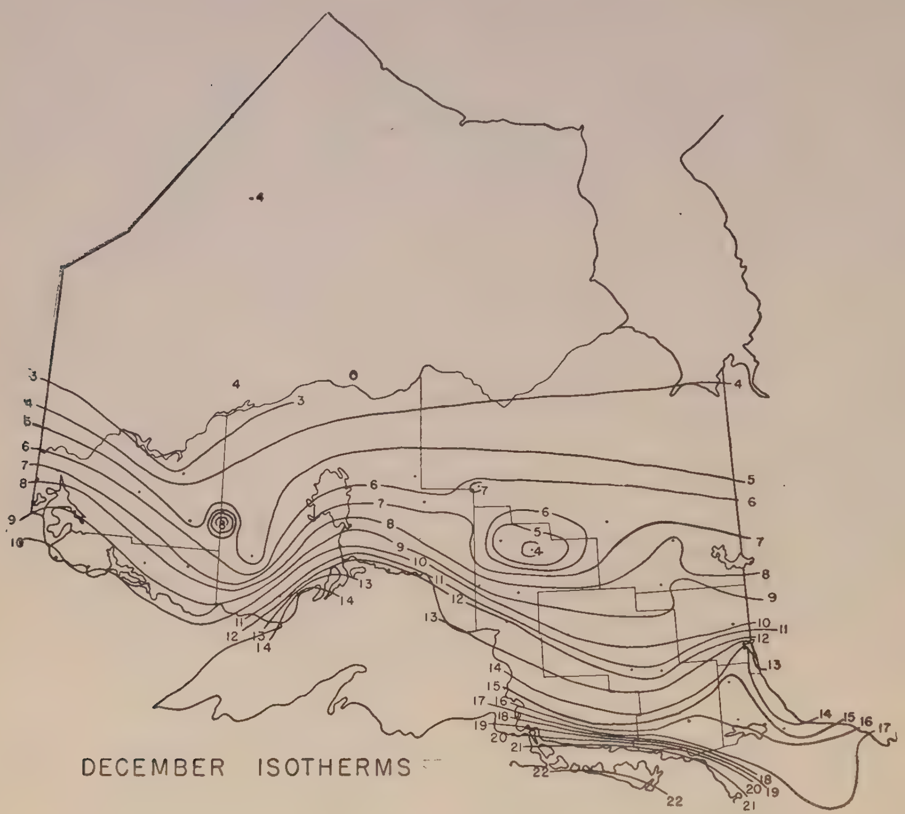


FIGURE 14

Lookout, Quorn, Savanne and Atikokan all have normals of 25 inches. Two Minnesota stations, Beaudette and International Falls, near Rainy River and Fort Frances, have records of 21 and 23 inches, the latter being 4 inches less than the normal for Fort Frances. As will be shown later when evaporation is considered, the Lake of the Woods area is the driest part of northern Ontario even though the least precipitation, 20.5 inches, is recorded at Moose Factory on James Bay. Ignace has a record of 32 inches and is the centre of a zone of higher rainfall and snowfall. On the railroad, the stretch between Dyment and English River is well known as a snow belt, but the northern and southern limits of this wetter zone are not yet defined.

Throughout northern Ontario there is a winter minimum and summer maximum in precipitation. This is most pronounced in Lake of the Woods area, Kenora having a February average precipitation of 0.92 inches and a July average of 4.04 inches. It is also strongly expressed at Kapuskasing and Cochrane where figures of 1.0 inch and 3.5 inches apply to February and July. September rainfall is equally high at these two stations in the Northern Clay Belt. The coincidence of warmth and rain in midsummer is perhaps the best feature of the climate as it affects agriculture and forestry. The light rainfall in April and May is favourable as it tends to allow the land

FIGURE 15. Lowest temperature on record.

to dry up so that it may be cultivated. The fairly heavy precipitation in September, however, often makes grain harvesting difficult and results in muddy conditions underfoot.

Snowfall

The variation in snowfall, as shown in Figure 26, is quite similar to that of total precipitation. Helen Mine with 174 inches has the heaviest snowfall while the least in northern Ontario, 43 inches, is recorded at Port Arthur. The northern reaches of the province and the Rainy River section receive less than 60 inches annually, on the average. The fact that the colder weather of northern Ontario keeps more snow on the ground would lead one to expect higher snowfall than in southern Ontario and it may come as a surprise to find that Cochrane and Ottawa have the same amount of snowfall. Furthermore, White River receives less snow than Owen Sound, Walkerton or Mount Forest; and Port Arthur, less snow than Toronto.

Rainy Days

The number of days with 0.01 or more inches of precipitation in a year or in a season does not vary in strict proportion to the amount of rainfall. In the southern part of the province, Barrie with only 31 inches of precipitation has an average of 188 days with rain or snow, the highest number of any station in Ontario. Stratford, with a normal precipitation of 38 inches, has an average of 97 rainy days a year. In northern Ontario,

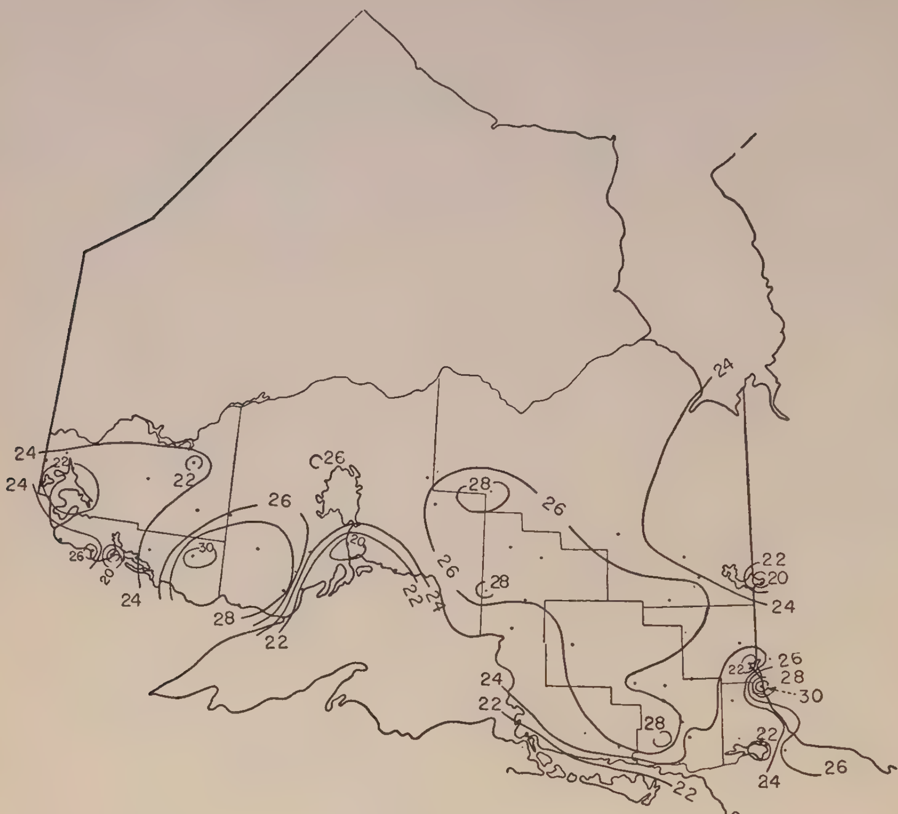


FIGURE 16. Mean daily range of temperature in July.

the number of rainy days in a year varies from about 150 in the east to about 85 in the west (Figure 27). Montreal River during the period of 1919 to 1939 had 159 days annually with 0.01 inches or more of precipitation while Kenora during the same period had 77 days annually with rain or snow. The average at most of the centrally located stations is about 125 days. Helen Mine, the station with the greatest precipitation, has an average of 134 rainy days a year.

During September, which usually is within the harvesting period, the average number of rainy days is 13 or 14 in the Temiskaming and the Northern Clay Belt and 8 or 9 in the Rainy River district. In other words at least two days in three are fine in the latter area while nearly every other days is rainy in the former.

Thunderstorms

Thunderstorms are not so frequent in northern Ontario as in the southern part. They are infrequent in the extreme north, judging by the Churchill Station which recorded 4.1 annually between 1937 and 1948. Helen Mine recorded only 4.5 thunderstorms per year. At many of the stations thunderstorms occur about 15 times a year. They are most frequent at Kenora and Sioux Lookout where 26.6 and 23.7 per year were recorded during the 1937–1948 period.

FIGURE 17. Start of growing season.

Hailstorms

The average number of hailstorms recorded in northern Ontario varies from 0.3 to 2.8 per year. The latter figure is for North Bay and it is the highest in the province. Cochrane recorded an average of 2 hailstorms annually from 1937 to 1948, but Kapuskasing and Timminins which are also in the Northern Clay Belt recorded only 0.6 and 0.8 respectively. Armstrong with 0.9, Sioux Lookout with 1.9 and Kenora with 0.6 give some idea of the number of times hail falls west of Lake Superior. An average annual number of 0.3 was recorded at Helen Mine.

Moisture Balance—Surplus and Deficiency

There has been given already a map of water need (potential evapotranspiration) which was used as a heat index. By comparing the computed monthly figures for water need with precipitation the amount of the excess or deficiency of soil moisture was obtained for each station according to the Thornthwaite method (7). On two maps, Figures 28 and 29, the annual surpluses and deficiencies are plotted.

The map of water surplus shows clearly the increase in wetness from west to east in northern Ontario, the amount of surplus being 4 inches on the Manitoba border and 12 inches on the Quebec border. The former is approaching the boundary of the prairie. The moist belt lying on the

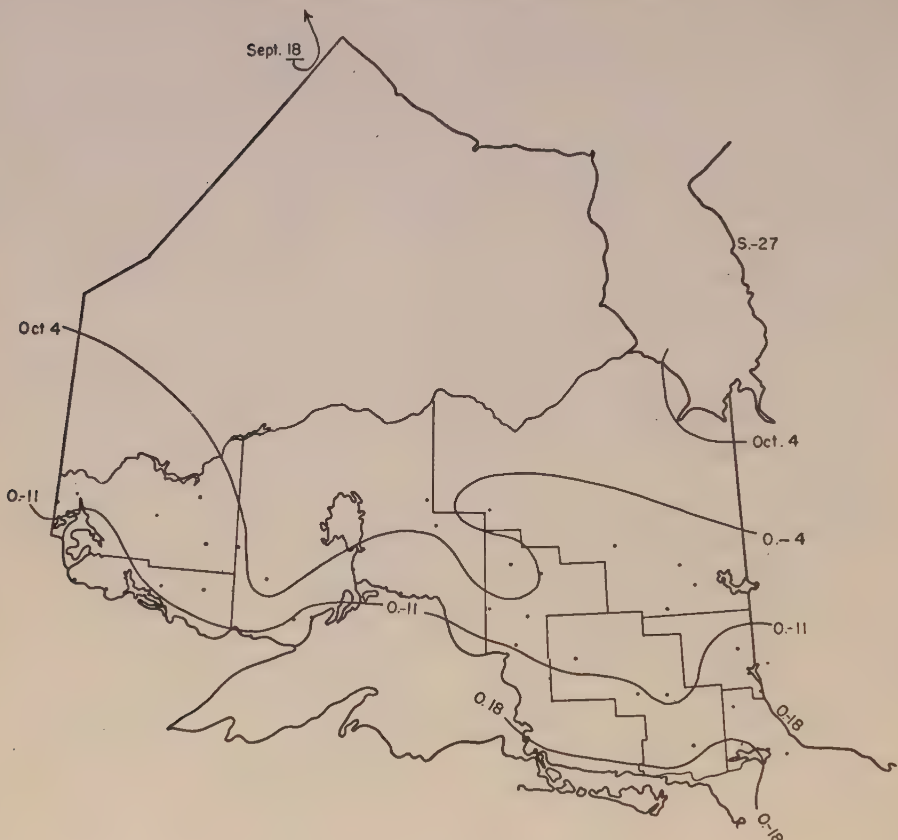


FIGURE 18. End of growing season.

slope east of Lake Superior and the weak rain shadow east of the height of land are again apparent as they were on the precipitation and snowfall maps. The 22-inch surplus in the Michipicoten area is based on a single station at Helen Mine but this record has been carefully checked and is believed to be accurate.

These quantities of water surplus may be compared with measurements of stream flow. While the gauging stations on the rivers and the weather stations seldom have operated during the same period, the average flow over 15 or 20 years may reasonably be compared to the average weather records. The measured stream flow is a little higher in northern Ontario than the computed run-off but the trend from wetness in the east towards dryness in the west is confirmed. For example the average annual run-off from the Abitibi River basin above Iroquois Falls during the period of 1920 to 1939 was 16.7 inches while the computed run-off at Iroquois Falls is 12.9 and at Amos, Quebec, 13.0. In the Rainy River region the Turtle River above Mine Centre, according to the 1914 to 1943 records, carries down 8.3 inches from the drainage area annually while the computed run-off at Mine Centre is 6.3 inches. Other records could be quoted which show that streams near the Quebec boundary carry off about 8 or 9 inches more water from their basins than those near the Manitoba boundary (5).

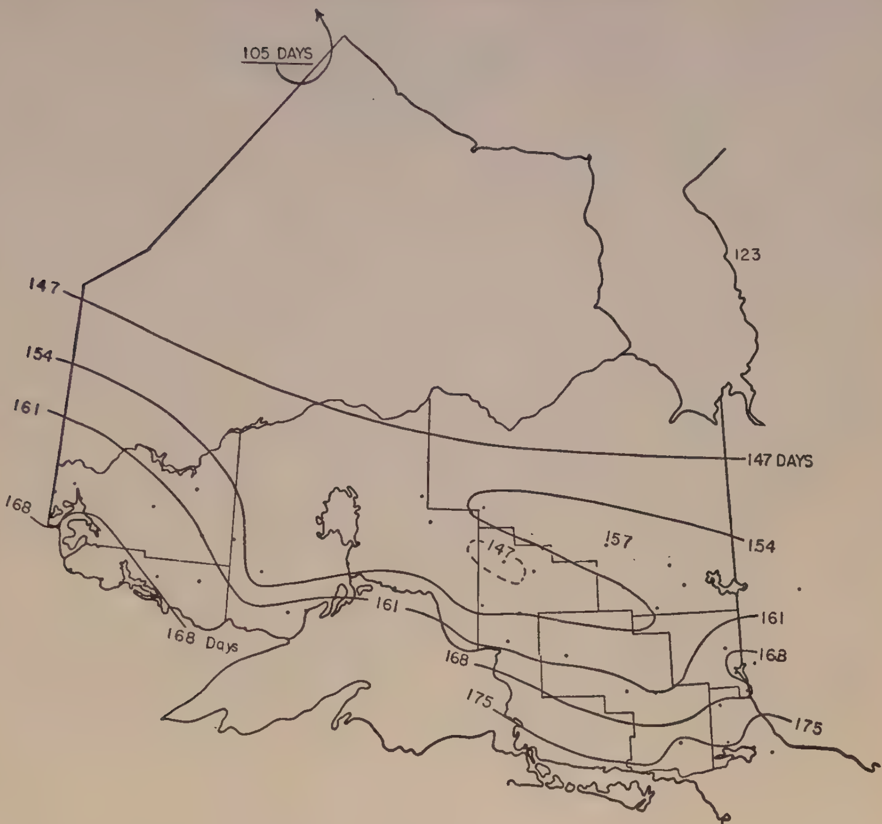


FIGURE 19. Average length of growing season.

A surplus of rainfall means muddiness, and in this respect the Northern Clay Belt, the Temiskaming Clay Belt and the clay pockets around Lake Nipissing suffer the most. Conditions underfoot, especially where the soil is heavy, have a strong bearing on the day-to-day life of the community. Construction is difficult under muddy conditions and work on the land is prohibited. Good roads are hard to maintain. The type of soil development is conditioned by the amount of surplus water; peat accumulation, podsolization and glei formation being promoted by a moisture surplus. In view of the frequency of surplus moisture the advantage of high sites and lighter, more pervious soil is readily seen.

In general, the map of moisture deficiency is the converse of the surplus moisture map. Moose Factory on James Bay, Abitibi Canyon and Iroquois Falls have no deficiency while it is computed to be 0.1 inches at Cochrane. Armstrong and Cameron Falls in the Lake Nipigon area show no deficiency. Other stations having zero deficiency are Crystal Falls, Helen Mine, Ignace and Mine Centre. Sudbury and Coniston show deficiencies between 2.5 and 3.0 which mark this as one of the drier areas of northern Ontario. Two stations close to the Manitoba border, Pinawa and Shoal Lake, show deficiencies of 3.3 and 2.3 inches while Kenora, a little to the east, has 0.8 inches. Kenora, in fact, has the most nearly ideal moisture regime of any point in Canada; that is the supply most nearly coincides with the need (5).

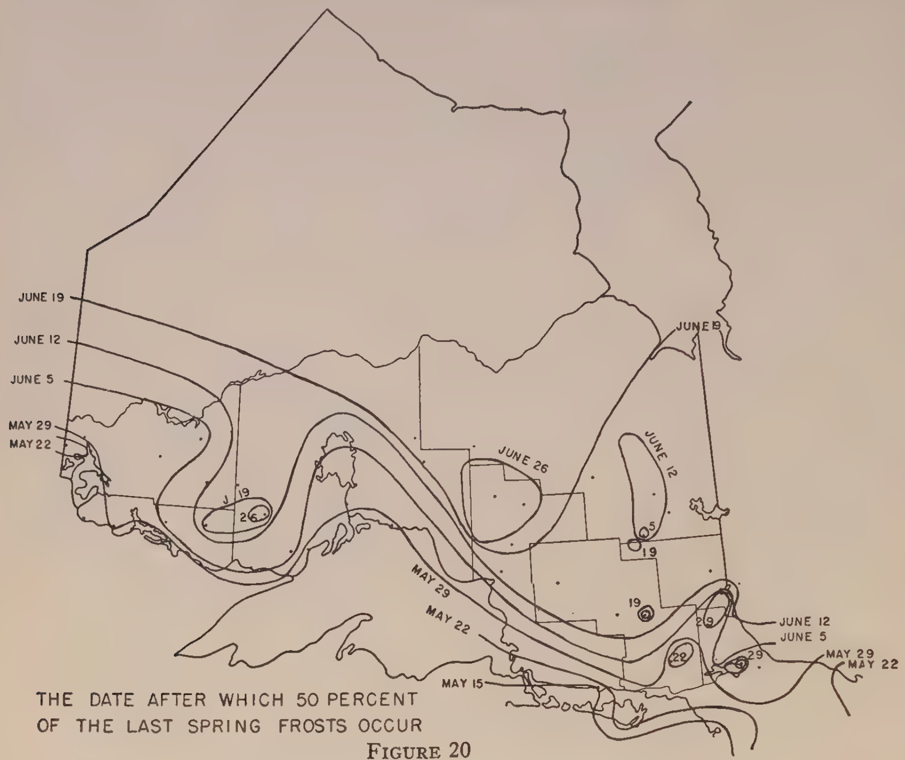


FIGURE 20

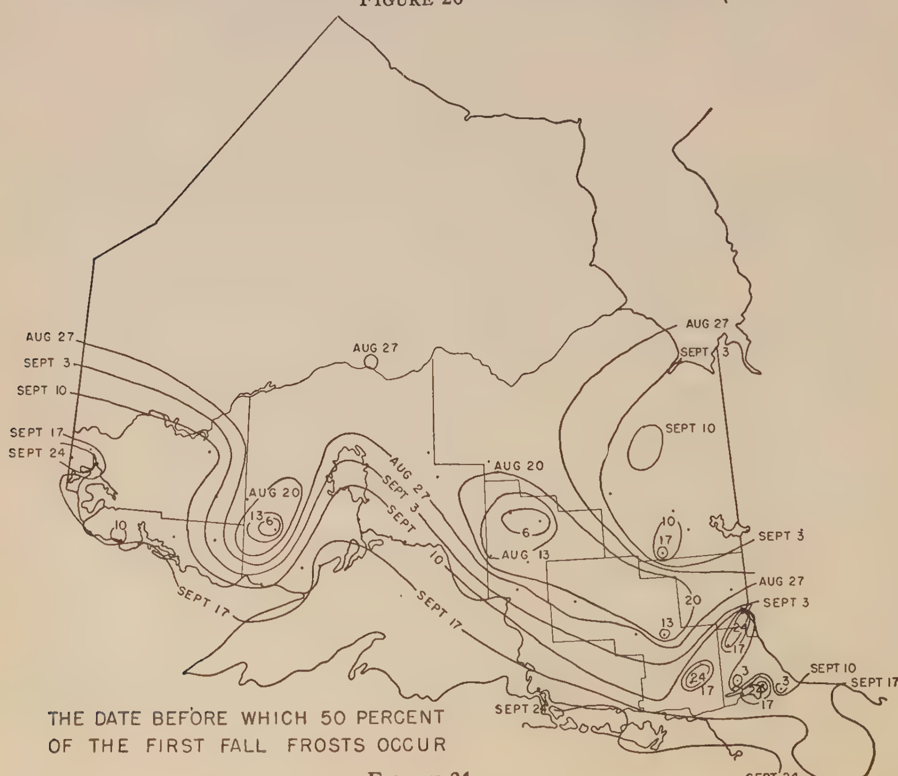
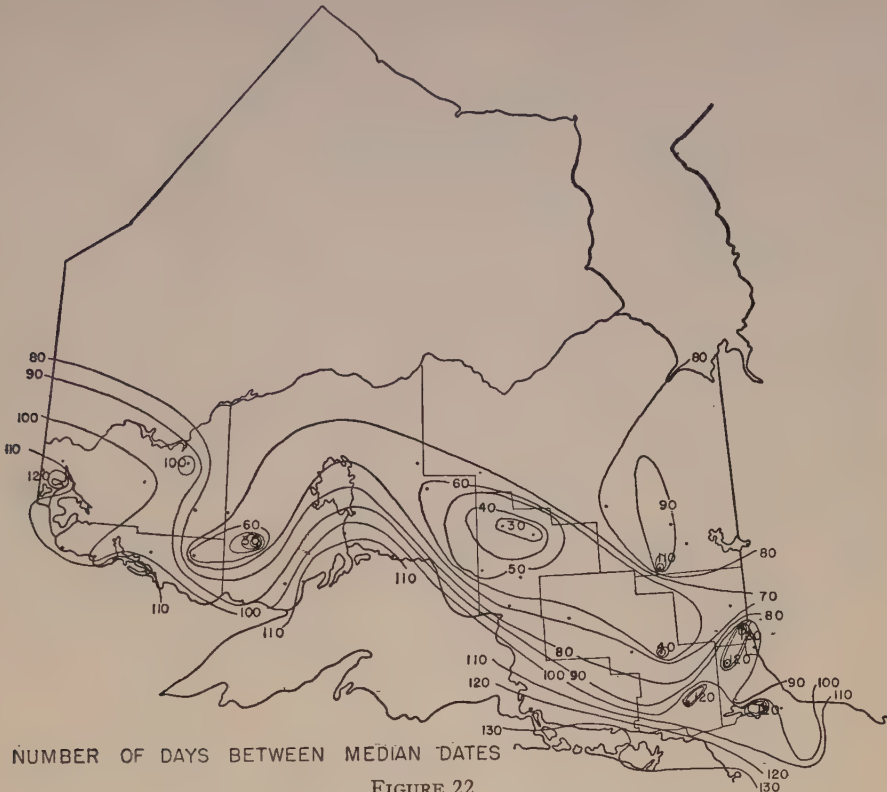
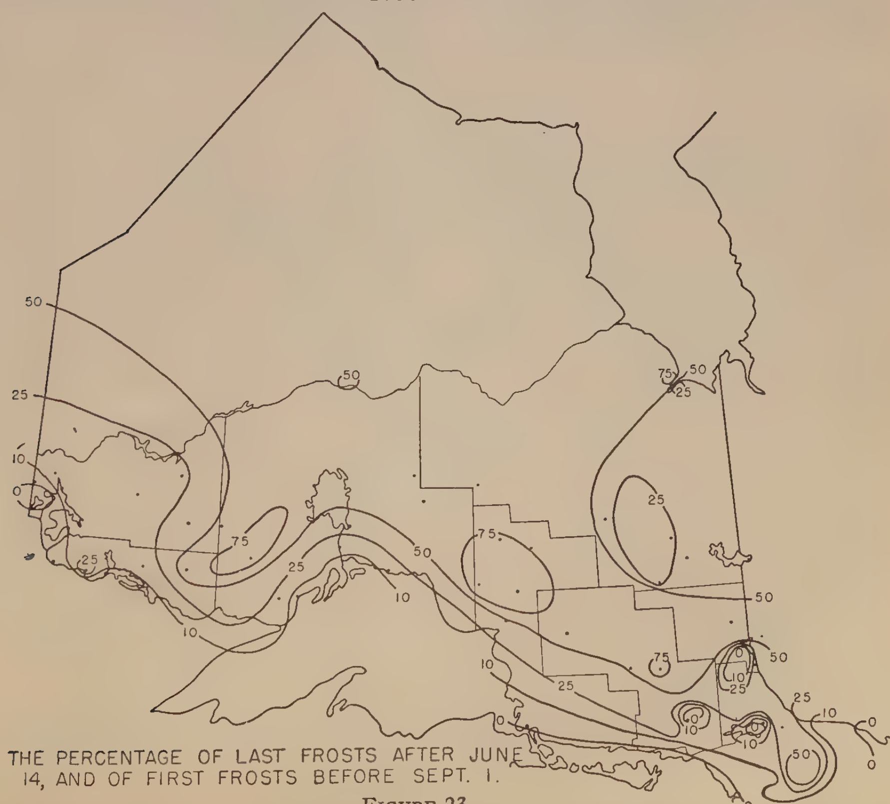


FIGURE 21



NUMBER OF DAYS BETWEEN MEDIAN DATES

FIGURE 22



THE PERCENTAGE OF LAST FROSTS AFTER JUNE 14, AND OF FIRST FROSTS BEFORE SEPT. 1.

FIGURE 23

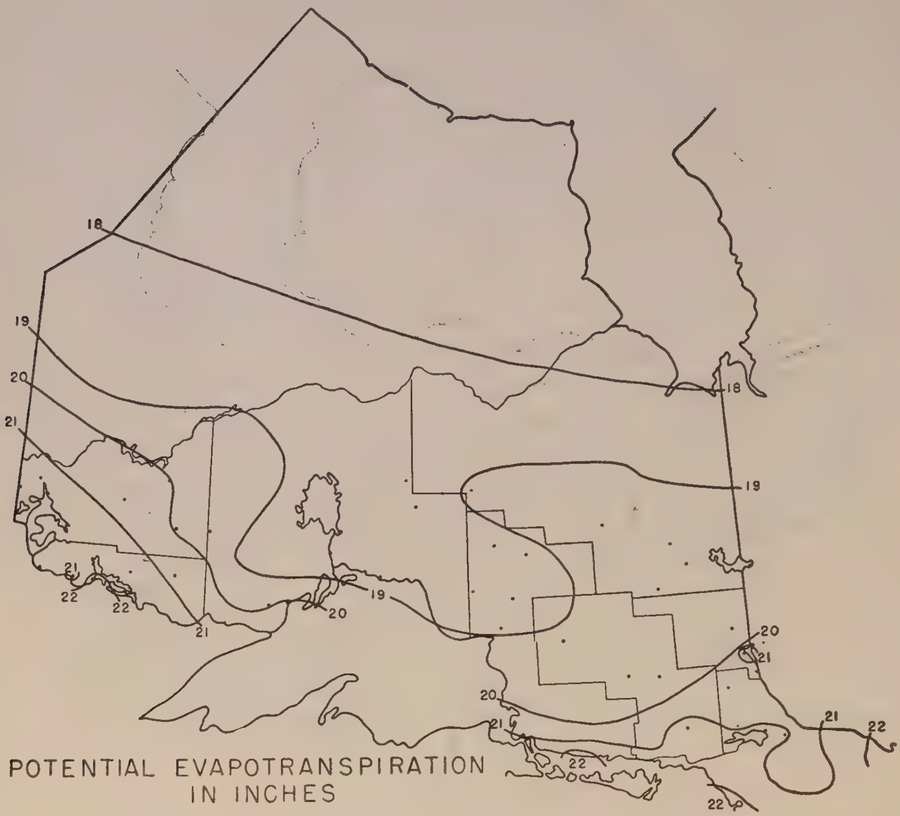


FIGURE 24

Frequency of Droughts and Surpluses

The figures just given on the moisture balance are all based on average weather data. They serve to compare the various parts of the country and to estimate average conditions. Beyond this, however, the seasonal variations from the normal should be known. Day-by-day accountings of soil moisture levels may be made according to the Thornthwaite formula but the daily computations are time-consuming and were only carried out for Kenora and Kapuskasing for 29 years. Kenora has a good record to represent the drier Lake of the Woods region while the record taken at the

TABLE 1.—NUMBER OF YEARS DURING 1921–1949 WITH SURPLUSES OF VARYING AMOUNTS

Surplus	Kapuskasing	Kenora
0– 4.9 inches	2	7
5– 9.9 inches	5	19
10–14.9 inches	12	3
15–19.9 inches	10	0

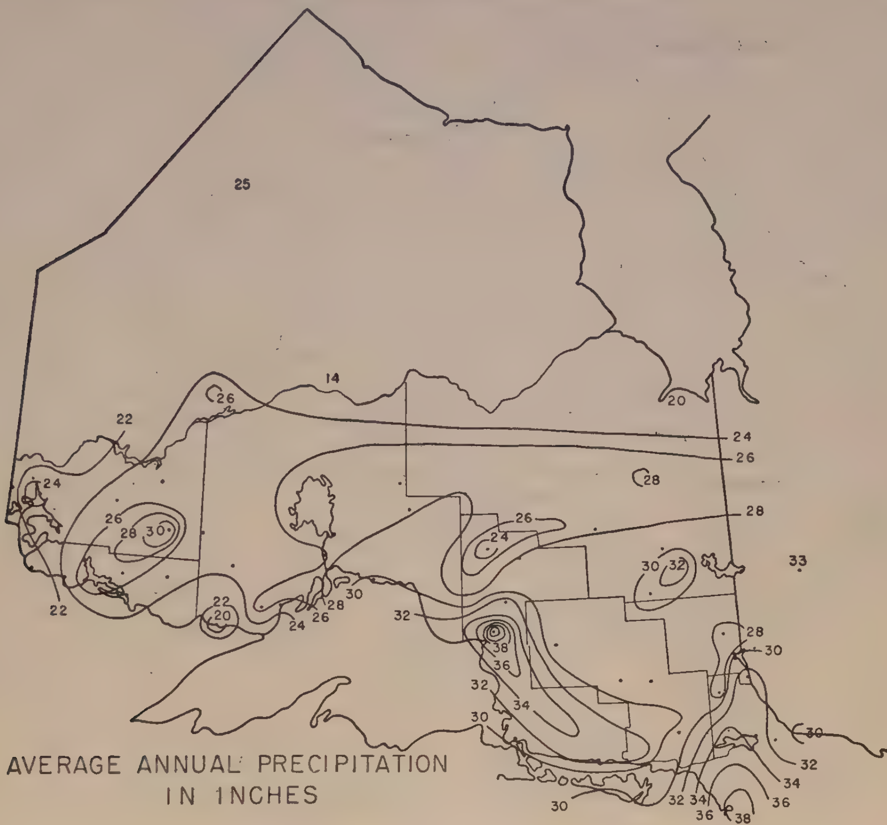


FIGURE 25

Dominion Experimental Station at Kapuskasing is representative of the moister eastern sections of northern Ontario. They may be compared with the results of a similar study made of Ottawa, Guelph, Walkerton and Harrow in the southern part of the province (6).

Table 1 affords easy comparison of the amounts of surplus precipitation during the 1921 and 1949 period. The occurrence of larger surpluses more frequently at Kapuskasing than at Kenora is appreciable and adds further data to stress the contrast between the climate of the Northern Clay Belt and the Lake of the Woods region.

TABLE 2.—OCCURRENCES OF MONTHLY DEFICIENCIES OF VARYING MAGNITUDE DURING THE PERIOD 1921–1949 AT KAPUSKASING AND KENORA

Amount of Deficiency (inches)	June		July		Aug.		Sept.		Oct.		Nov.	
	Kap.	Ken.	Kap.	Ken.	Kap.	Ken.	Kap.	Ken.	Kap.	Ken.	Kap.	Ken.
0	23	20	10	11	8	5	18	11	27	17	29	27
0–2	6	7	16	10	15	9	11	16	2	12	0	2
2–4	0	2	3	7	6	13	0	2	0	0	0	0
4–6	0	0	0	1	0	2	0	0	0	0	0	0

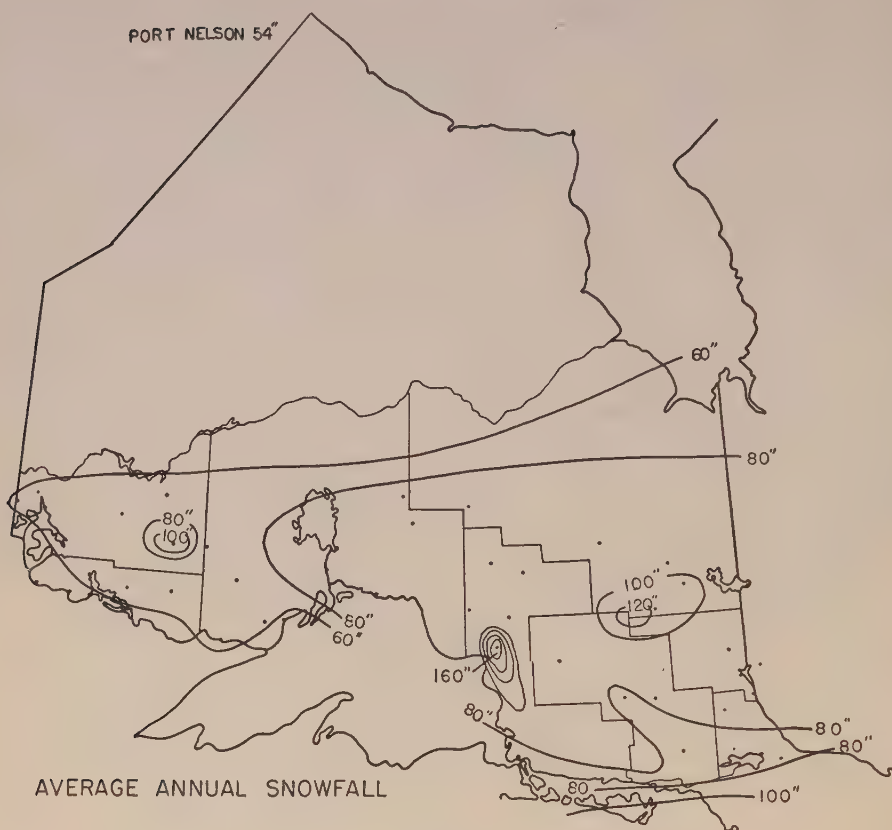


FIGURE 26

Another table, Table 2, lists the number of deficiencies of various magnitudes, according to the Thornthwaite method, occurring at Kapuskasing and Kenora. In spite of the moist climate at Kapuskasing there were deficiencies in 24 of the 29 years studied but most of them were of a small order, only four seasons having more than 4 inches. The record at Kenora showed droughts in 26 of the 29 years and in 10 of these the moisture deficiency was over 6 inches. More than half of these deficiencies at both stations occurred in August and a high proportion in July and August together. Occasionally, small deficiencies of soil moisture happen in June and October, especially in October at Kenora.

WINDS

The direction and speed of winds are measured at the airports and a few other weather stations. Climatic Summaries, Volume 2 (1) gives tables of average values for speeds from the eight main compass points at 14 Ontario stations and also gives the percentage of winds blowing from each direction. Wind speeds are mostly from 8 to 12 miles per hour in winter and 6 to 10 miles per hour in the summer. Northwest winds are most frequent in winter; they amount to over one-quarter of all winds at some stations. West and north winds are also common. In midsummer south-

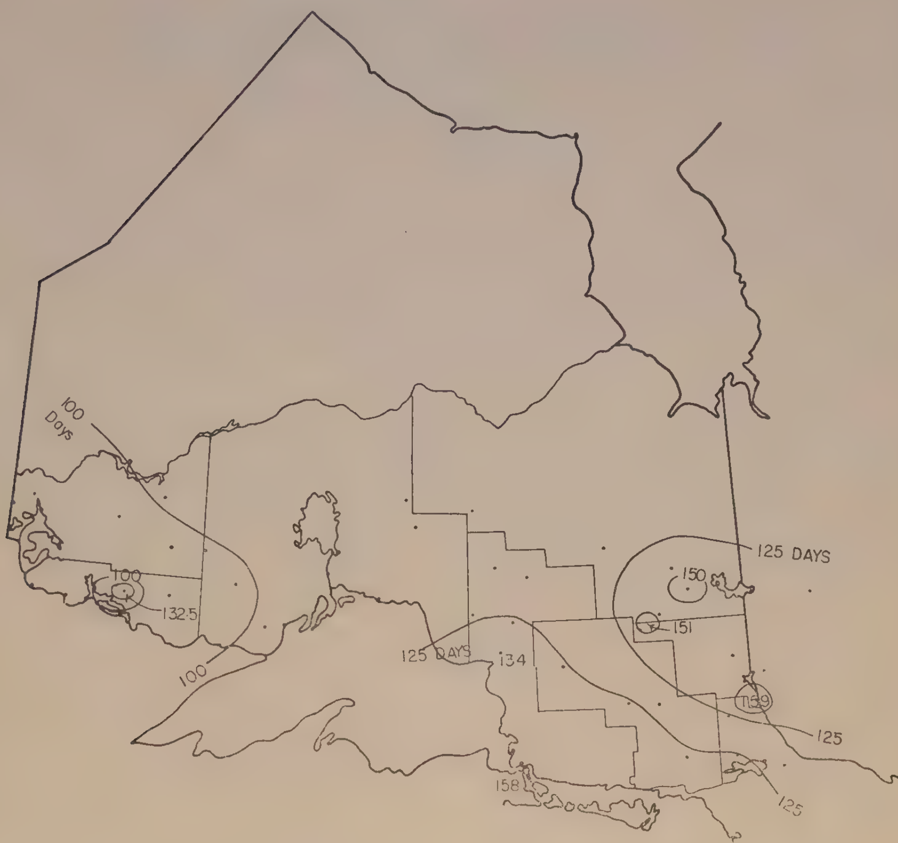


FIGURE 27. Number of rainy days in a year (1919–1939).

west winds prevail in northern Ontario while west or northwest winds are most frequent at some stations. East winds are least frequent and these are also the gentlest ones. Wind speeds of over 30 miles per hour are not frequent at any time of the year and 40-mile-an-hour winds occur only on rare occasions.

SUNSHINE

In northern Ontario five stations, Armstrong, Moosonee, Kapuskasing, New Liskeard and Turbine have records of bright sunshine for extended periods. The Winnipeg record may be consulted also for indications of conditions in the Kenora-Rainy River districts. The results for these stations, stated as per cent of possible sunshine per month, are shown in Table 3.

The sunniest weather is in July and August, except at Moosonee where June is the brightest month. The dullest weather comes in November and December, the lowest recorded figure being 14 per cent of the possible sunshine at New Liskeard in November. Moosonee receives the least sunshine of these five places and Turbine the most, indicating a trend

TABLE 3.—AVERAGE SUNSHINE—PER CENT OF THE POSSIBLE DURATION

Station	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Armstrong	37	43	42	44	39	42	51	50	36	33	19	27
Kapuskasing	30	37	40	40	45	47	48	45	35	25	18	21
Moosonee	31	40	35	38	41	47	44	40	29	20	18	17
New Liskeard	22	32	34	42	40	43	43	46	34	22	14	19
Turbine	24	36	38	44	51	51	58	58	44	35	21	22
Winnipeg	38	46	46	50	52	52	60	59	47	38	32	31
Toronto	27	37	41	46	50	58	62	60	54	44	30	24
Harrow	29	33	34	41	56	58	65	62	50	47	33	21

towards less cloudiness in the south. Winnipeg gets a little more sunshine than Turbine in summer and considerably more in winter. Armstrong supports Winnipeg in pointing out the sunny winter weather in the western part of northern Ontario. In fact there is considerably more sunshine at Armstrong in January (37 per cent) and February (43 per cent) than at Toronto, St. Catharines or Harrow.

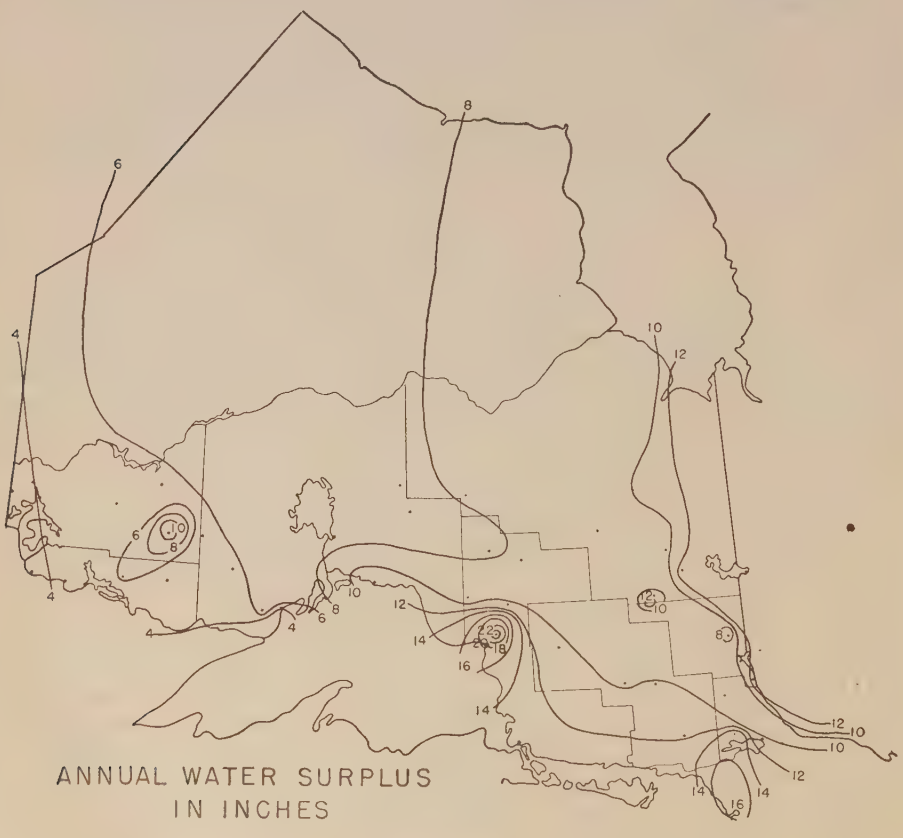


FIGURE 28

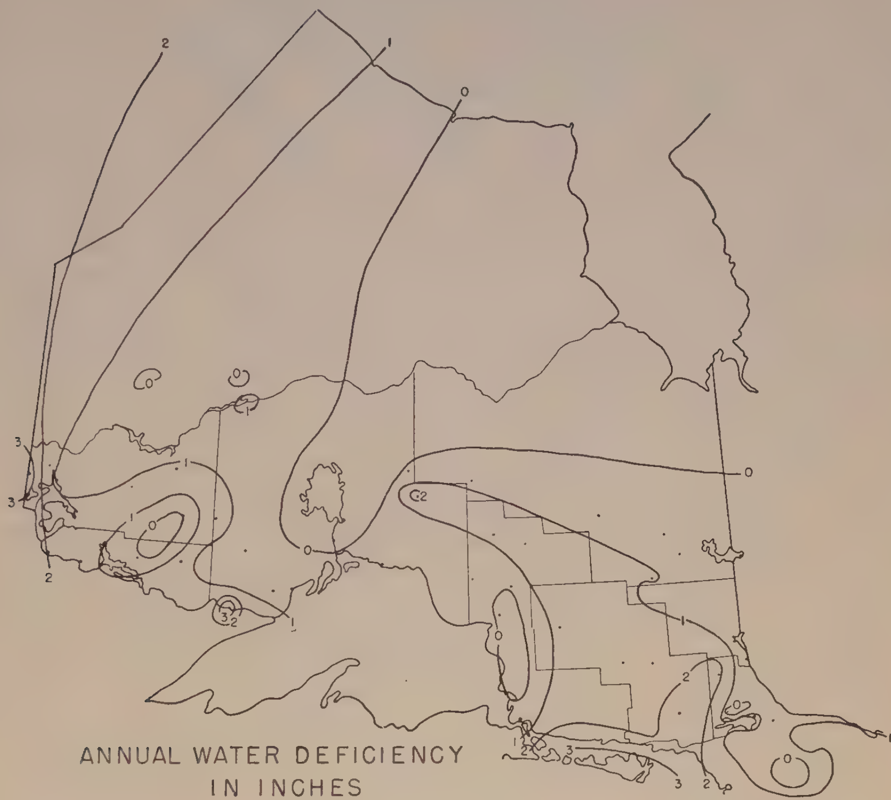


FIGURE 29

HUMIDITY

Humidity records are taken at only a few principal stations, mostly at airports, and until recently the measurements in winter when temperatures are below freezing were subject to error. Climatic Summaries, Volume 2 (1) gives short-term humidity records for seven northern Ontario stations while the Sault Ste. Marie, Michigan, station can also be used. The records of Winnipeg, The Pas and Churchill in Manitoba are also valuable in relation to the western and far northern parts of the province. Dew points, relative humidities and mixing ratios (grains of water vapour per kilogram of dry air) are given for these few stations.

Figures 30 and 31 are maps showing the variations in mixing ratios in January and July respectively. They are copies of the Ontario section of Canadian maps drawn by Boughner and Thomas in the above-mentioned Climatic Summaries. In both winter and summer there is less water vapour in the air in the north than in the south, the amount of difference being greater in the summer, but the percentage difference being higher in winter. For example there are 1.1 grams of water per kilogram of dry air in January at North Bay and 0.7 at Kapuskasing, the latter being 62 per cent of the former. The average mixing ratios in July at North Bay and

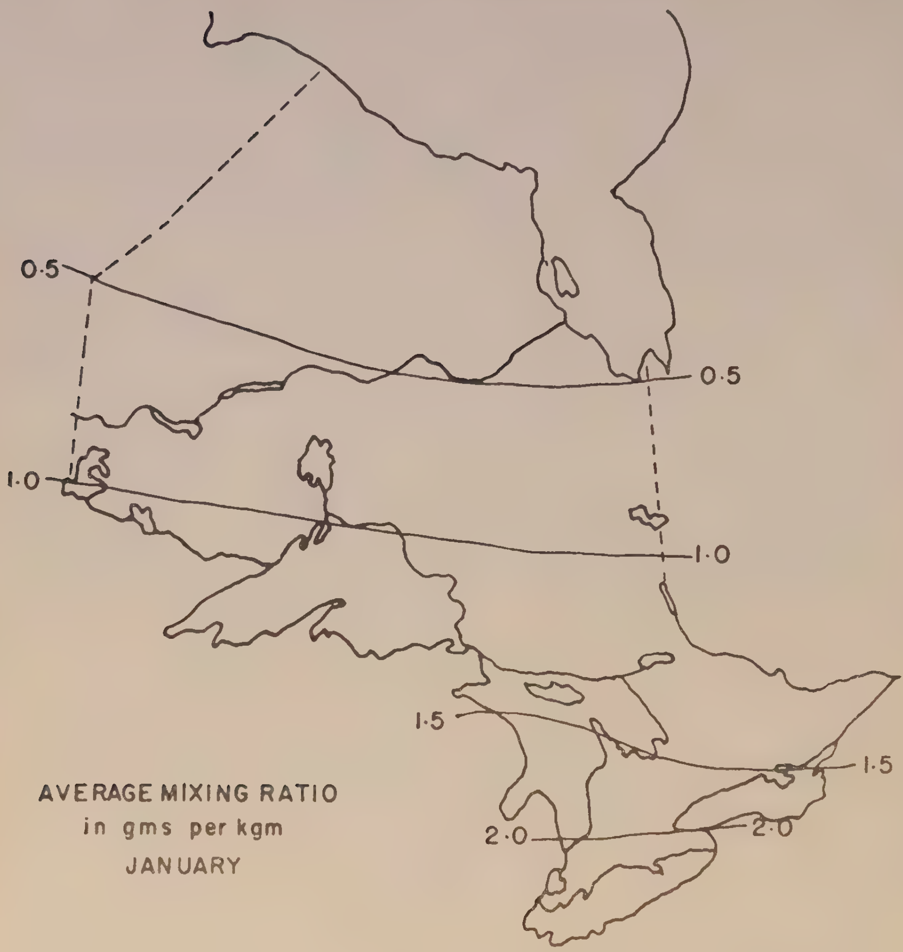


FIGURE 30 *(Meteorological Division, Canada Dept. of Transport)*

Kapuskasing are 10.3 and 9.1, the latter being 88 per cent of the former. The dew point temperatures vary accordingly, being lower in the north. The air is driest in January and contains the most moisture in July.

The other common method of expressing the moisture content of the air is as relative humidity. This is the mass of water vapour, stated as a percentage of the maximum amount, that the air will retain at the existing temperature and pressure. Unlike the mixing ratio, the relative humidity varies widely due to either daily or seasonal temperature fluctuations. In winter in northern Ontario it varies in the opposite manner to the mixing ratio. At most stations it is higher in the north than in the south and might mistakenly give the impression that there is more moisture in the air in the north if the mixing ratios were not given as well. There is no regular trend from north to south or from east to west in summer. The average relative humidities for eleven stations in winter, spring, summer, and fall are given in Table 4. The relative humidity is lowest in April or May and highest in December, January, or February.

TABLE 4.—AVERAGE RELATIVE HUMIDITY

Station	Dec. Jan. Feb.	Mar. April May	June July Aug.	Sept. Oct. Nov.
Churchill			81	91
The Pas	88	74	71	80
Winnipeg	91	73	70	79
Sioux Lookout	89	69	74	83
Fort William	87	74	80	84
Nakina	93	73	74	87
Kapuskasing	90	75	74	86
Porquis Junction	92	75	73	85
North Bay	89	75	76	83
Sault Ste. Marie	83	75	77	82
Malton	83	74	73	79

COMPARATIVE CLIMATES

According to Koppen's classification (2) northern Ontario has two types of climate, the boundary being near latitude 50°, 30′, which is north of all the settlements except Moosonee. North of this line there is a Cool Snow Forest climate with cool summers (D f C) while to the south it is classed as Cool Snow-Forest with warm summers (D f b). The latter applies to all southern Ontario. Considerably finer definitions are provided by Thornthwaite's classifications. His original system (7) (8) shows three types of climate in northern Ontario. Practically the whole area between Lake Superior and Hudson Bay is classed as Taiga (D′). A belt north of Lake Huron and extending into Temiskaming falls into the Humid, Microthermal class (B C′ r). The Thunder Bay district also falls into this class, but the Rainy River area, being slightly drier, is classed as Sub-humid Microthermal (C C′ r). The more recent Thornthwaite classification divides the area differently with respect to temperature and recognizes five moisture zones, the driest, in the Rainy River area, being Sub-Humid (9). Helen Mine with a Perhumid rating represents a sixth class.

It is generally conceded that no two separate regions of the earth have precisely the same climate but it is desirable to know the nearest approaches to the local climate, particularly of the settled areas. A narrow belt across southern Norway and Sweden has a climate resembling that of the warmest parts of northern Ontario, but the winters are not as cold. This belt continues from the Baltic States half-way across Russia. The winters are more severe eastward and some of the stations near the Ural Mountains compare nicely with local stations. Sverlovsk, U.S.S.R., and Cochrane, Ontario, have normal January and July temperatures of 2° and 0° F., 63° and 63° F. respectively. Similarly, Moscow and Sudbury have temperatures in January and July of 12° and 10° F., 66 and 66 degrees. However the Russian region is drier and thus compares more closely with the Rainy River-Kenora area. Another smaller area similar to the warmer parts of northern Ontario is found in the far east of Asia bordering the Tartar Strait on the mainland and on the island of Sakhalin, although the precipitation there is slightly higher.

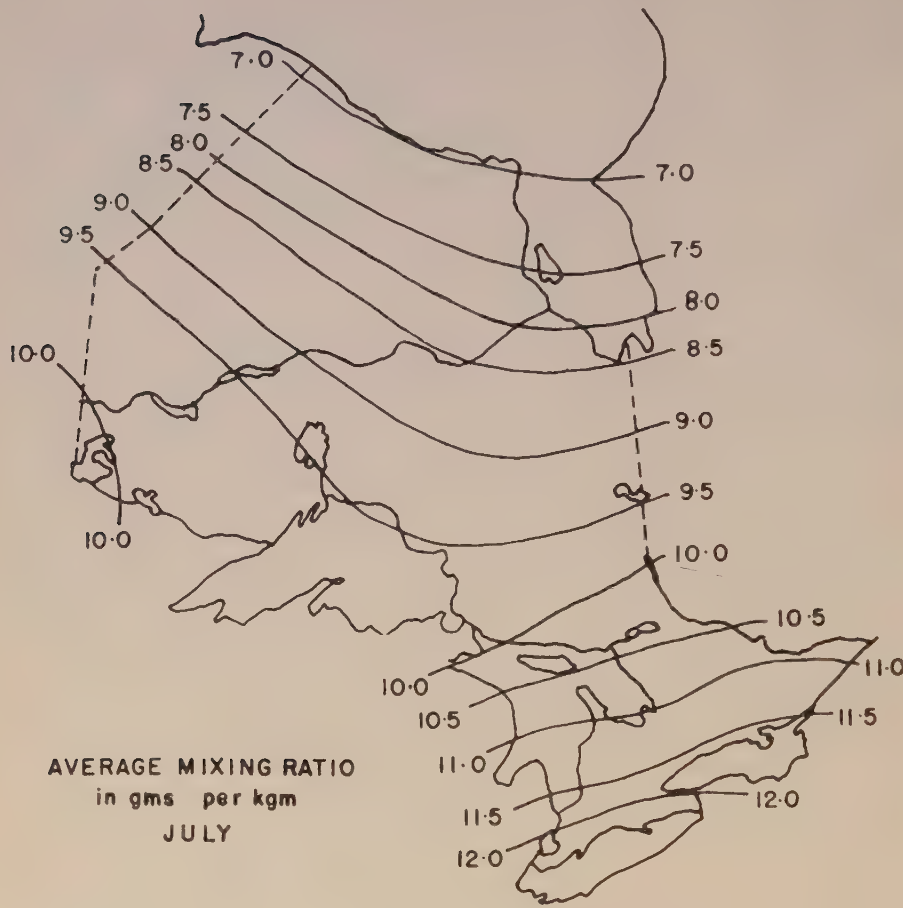


FIGURE 31 *(Meteorological Division, Canada Dept. of Transport)*

A narrow belt of Thornthwaite's humid microthermal (B C′ r) climate is mapped on the coastal range of British Columbia, extending south into Washington and Oregon. However the winters are much milder in this area than in any part of Northern Ontario. The two British Columbia stations most closely approximating the warmer parts of northern Ontario are Golden in the north Columbia River valley and Greenwood in Kettle valley. The former is almost the same as Sault Ste. Marie. The latter has warmer winters corresponding to those found at Barrie or Kingston. The precipitation at these two stations in the growing season is less than half that of the Rainy River area, the driest part of northern Ontario.

CLIMATIC REGIONS

In describing the climate of southern Ontario the part south of North Bay was divided into 15 regions. Such a treatment, although arbitrary, serves to collate and summarize the characteristics of climate throughout an area. In northern Ontario there are few records for the section north of the Canadian National Railway, so that part at most can be divided only by an east-west line on the basis of temperature and possibly by a north-

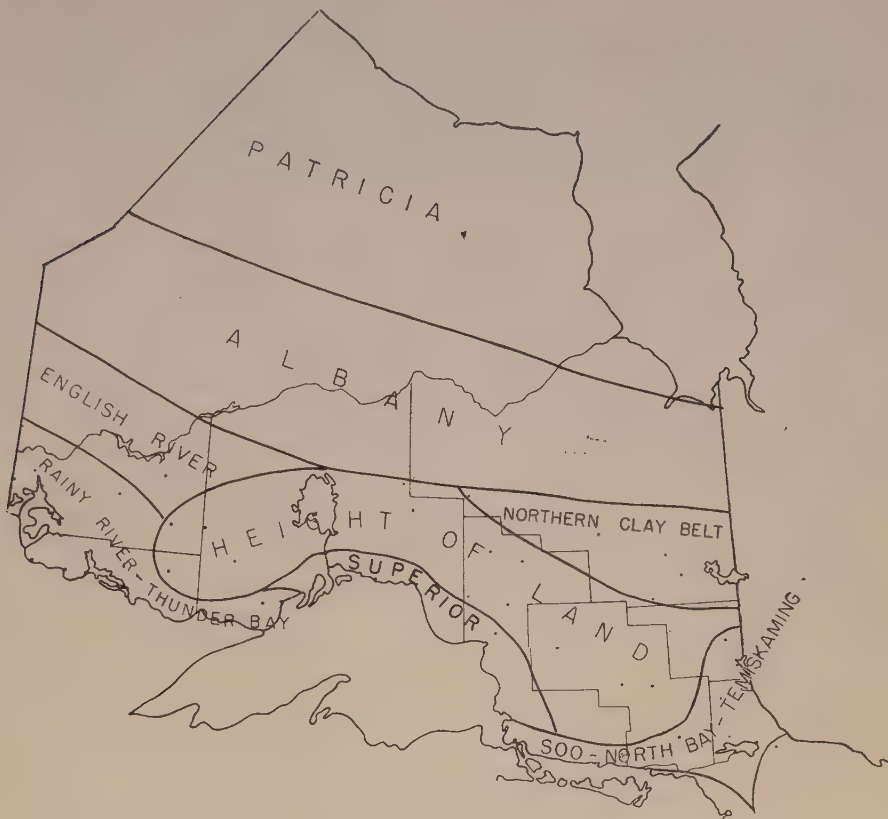


FIGURE 32. Climatic regions of northern Ontario.

south line on the basis of moisture. The two warmest sections, lying east and west of Lake Superior on the southern fringe, must be separated from the rest of the area. The Rainy River-Thunder Bay section would be separated on the basis of dryness as well as heat. The Temiskaming Clay Belt is included with the North Bay-Sault Ste. Marie belt because it is more like that area than the higher, rocky country adjacent on the west. This leaves the Northern Clay Belt and the rocky belt over the height of land north and east of Lake Superior. The shoreline belt, ameliorated by Lake Superior, may be separated and this can be expanded to include Helen Mine with its high precipitation. The important difference between the Clay Belt and the Height of Land is in the frost-free period. Perhaps some of this difference is due to the settings of the opposing stations. At Kapuskasing, Cochrane and Iroquois Falls the records are taken on high ground in cleared country while such stations as Chapleau, White River, Hornepayne and Savanne are situated in valleys surrounded by rocky, wooded country. There is probably more difference locally between high ground and wet, usually peaty, depressions than there is between these two regions. However, taking the weather data as they come, the Northern Clay Belt may be distinguished from the Height of Land and is treated as a separate region. Similarly, another division is made in the west, north of the Rainy River area, because of a longer growing season.

Figure 32 is a map of climatic regions along the lines discussed above, except that Patricia and Albany are not divided into moister eastern and drier western portions. The characteristics of these eight regions are summarized in Table 5. This table gives representative values of the most important climatic characteristics and affords an easy comparison of the different sections of northern Ontario.

TABLE 5.—REGIONAL CLIMATES

	Temiskaming, North Bay, Sault Ste. Marie	Rainy River, Thunder Bay	Superior	Northern Clay Belt	English River	Height of Land	Albany	Patricia
Altitude	600–1000	600–1400	600–1400	700–1000	1000–1500	1000–1600	0–1500	0–1500
July Normal Temperature	66	67	61	63	65	61	62	59
January Normal Temperature	10	3	7	−1	−3	1	−5	−10
Extreme Low Temperature	−54	−60	−54	−73	−57	−63	−57	
Extreme High Temperature	106	108	102	103	105	108	100	102
Daily Range of Temperature in July	23	23	23	24	24	27		
Dates after which there are no frosts in 3 out of 4 years	May 30	June 5	June 15	June 20	June 10	July 1		
Dates before which there are no frosts in 3 out of 4 years	Sept. 17	Sept. 10	Sept. 1	Sept. 1	Sept. 1	Aug. 10		
No. of days between the above frost dates	110	100	90	75	85	40		
Beginning of Growing Season	April 25	April 28	May 5	May 7	May 1	May 7	May 10	May 20
End of Growing Season	Oct. 17	Oct. 15	Oct. 10	Oct. 8	Oct. 8	Oct. 6	Oct. 4	Sept. 30
Average Length of the Growing Season	175	170	160	157	160	155	150	135
Heat Units per year	21	21	19	19	20	19	18	15
Mean Annual Precipitation	30	24	35	29	25	27	23	20
Mean Annual Snowfall, inches	75	60	100	90	65	90	70	60
Average Rainfall April–Sept.30	18	17	18	17	17	16	14	13
Actual Evapotranspiration	20	19.5	18.5	18.5	19	18	17.5	15
Average Annual Moisture Def.	1	1.5	.5	.5	1	1	.5	0
Average Annual Moisture Surplus	10	5	16	10	6	9	8	8
Average Mixing Ratio in July	10	10	9.5	9.5	10	9.5	9	7.5
Average Mixing Ratio in Jan.	1.2	1.0	1.1	0.8	0.7	0.9		

ACKNOWLEDGMENTS

This study has been greatly aided by C. C. Boughner and his staff of the Meteorological Service of Canada, particularly in placing unpublished records at the disposal of the author. Much of the statistical work was done by H. Lochhead, E. Porter and Marie Sanderson of the Ontario Research Foundation.

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SEED SETTING OF ALFALFA FLOWERS TRIPPED BY BEES AND MECHANICAL MEANS[1]

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ABSTRACT

A study was undertaken to determine the effectiveness of a locally built mechanical tripper and of leaf-cutter bees, bumble bees and honey bees in bringing about tripping and seed setting in alfalfa. Automatic or mechanical tripping followed by self-pollination resulted in 31.6 per cent of pod formation, and 1.7 seeds per pod. When tripping was accompanied by cross-pollination 74.8 per cent of the flowers formed pods and these pods contained an average of 4.7 seeds. Bumble and leaf-cutter bees tripped 90.2 and 61.0 per cent, respectively, of the flowers under observation. In both cases, the pods contained on the average 4.5 seeds which is proof that these bees cross-pollinated when they tripped the flowers.

Honey bees were ineffective both as trippers and pollinators of alfalfa flowers. There was an increase in the incidence of automatic tripping when honey bees had been in a cage for a period of five days. The average number of seeds per pod under natural conditions was 4.4, proving that most of the pods set in the field were from flowers that were tripped and cross-pollinated. It is assumed that bumble bees and leaf-cutter bees were responsible for the tripping and cross-pollination.

A single passage over each of two fields with a tripping machine tripped 28.2 and 46.3 per cent, respectively, of the flowers that were open at the time. Of the tripped flowers, however, only a small proportion formed seed, and the number per pod was similar to that obtained from selfing. The machine was found to be ineffective in increasing alfalfa seed yields.

INTRODUCTION

Variability in seed setting continues to be the greatest problem in alfalfa seed production. There still are differences of opinion regarding the relative importance of automatic and insect tripping and of cross-pollination and self-pollination.

Brink and Cooper (3) have shown that fertilization can occur without tripping. Other workers (5, 13, 16), however, have shown that insignificant amounts of seed are produced from untripped flowers under field conditions.

The work of Tysdal (16), Knowles (8), Bolton (2) and Lesins (9) appears to be quite conclusive in demonstrating that a much higher percentage of pods, and up to three times as many seeds per pod, develop from cross-pollinated, compared with self-pollinated flowers. The work of Brink and Cooper (4) indicates that the lower seed set per pod from selfed flowers results from partial incompatibility and poorer growth of male gametophytes and high incidence of ovule abortion from inbred embryos.

If seed yield alone were considered, increasing natural seed set with mechanical trippers and subsequent self-fertilization might have some value. Work by Tysdal *et al.* (17) and Hadfield and Calder (5) shows, however, that self-ferilization causes a decrease in seed and forage yields of subsequent generations. Mechanical trippers have from time to time been tried with the hope of overcoming seed yield fluctuations. According to the review

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of Tysdal (16) and the reports of Silversides and Olson (14) and Jones and Olson (7) mechanical trippers have not been successful even in improving seed yields.

Until quite recently there was general agreement with Tysdal's conclusion (15) that wild bees were primarily responsible for tripping and seed setting in alfalfa. This was confirmed in Sweden by Akerberg and Lesins (1) who showed that under their conditions, bumble bees, leaf-cutter bees and honey bees tripped with 78 per cent, 100 per cent, and 0.83 per cent efficiency, respectively. Recently, however, alfalfa workers in Utah (18, 6, 12) and in California (10, 11) have reported that honey bees, particularly pollen gathering honey bees, were responsible for tripping and seed-setting under their conditions. Apparently, then, the part played by honey bees deserves further consideration.

Since alfalfa seed production is so important in large areas of northern Alberta, and since information on factors mentioned earlier was lacking for this area, a study was undertaken to determine the effectiveness of a locally built mechanical tripper and of leaf-cutter bees, bumble bees and honey bees in bringing about tripping and seed-setting in alfalfa.

MATERIALS AND METHODS

Test of Honey Bees, Leaf-Cutter Bees and Bumble Bees, and Controlled Selfing and Crossing

In order to study the influence on seed-setting of the various kinds of bees as compared with hand selfing and crossing, an experiment was conducted in a field at Tiger Lily. This location is in one of the good alfalfa seed producing areas on Alberta gray wooded soil, approximately 100 miles from Edmonton.

Control exposure of alfalfa to the different kinds of bees was made possible by the use of four wire screen cages, each $3' \times 3' \times 3'$, placed on the field during the latter part of June prior to flowering. The following treatments were included:

A. Natural environment—uncaged check.
B. Absence of bees in the cage.
C. Five honey bees in the cage.
D. Five bumble bees in the cage.
E. Five leaf-cutter bees in the cage.

The bees in C, D, and E were handled in the following manner: Actively working insects were captured and introduced into the cages. After 24 hours they were released and freshly captured ones were introduced. Unfortunately no attempt was made to identify the bees in D and E as to species, but only those found to be actively tripping were used.

In each treatment 25 racemes of fresh untripped flowers were tagged and the total number of flowers recorded prior to introduction of the bees.

For self-pollination the fresh flowers on 25 racemes were tripped on a clean toothpick. A fresh toothpick was used for each plant. Controlled cross-pollination was performed by collecting pollen from several different plants on toothpicks and tripping the fresh flowers of 25 racemes on these toothpicks.

Records were obtained of each flower in all treatments from flowering to wilting or pod setting. At maturity the racemes were harvested, the number of pods and the number of seeds in each pod were recorded.

The entire growth under each cage was harvested at maturity and yields of seed were obtained. The caged and uncaged check material was harvested and handled in a similar manner.

Tests with a Mechanical Tripper

The field scale mechanical tripper was made available for testing by the manufacturer. The principle of this machine was developed on a pilot model by H. Evjen, a farmer of the Stony Plain district. The Coutts Machine Co., Ltd., of Edmonton, manufactured a field-scale model in 1949-50. The principle is that of vertical, sponge rubber-covered rollers working in pairs. As the machine moves along, the top growth is passed between a pair of rotating rollers. The pressure and shearing force of the rollers accomplishes the tripping. The machine is illustrated in Figures 1, 2 and 3. The machine was tested at two different locations. One series of tests was conducted at Tiger Lily while the other series was conducted on black soil of the Edmonton area where normally little seed is set. Because of transportation difficulties, the tests with the mechanical tripper were not exactly comparable in both locations.

The following strip tests were conducted with the machine on the farm in the Edmonton area:

A. A single treatment.
B. Two treatments at different dates.
C. Two treatments on the same day.
D. Two treatments on one day followed by an additional one at a later date.
E. Three treatments on the first day.
F. Three treatments on the first day followed by an additional one at a latter date.
G. Three treatments at different dates.
H. Checks.

Two untreated check strips were left, one between treatments B and C, and one between treatments E and F.

Strips at Tiger Lily were treated as follows:

1. Check strip.
2. Once over.
3. Twice over.
4. Three times over.
5. Twice over at different dates.

Tripping and subsequent seed setting in machine-treated material were studied in the following manner: At Tiger Lily, prior to treatment with the machine, 25 racemes, from which all pods, tripped flowers and buds were removed, were tagged. Immediately after treatment the flowers tripped by the machine were counted and all untripped flowers were removed. At Edmonton, racemes were tagged and records taken only in the strips that had been treated once. In September, all tagged racemes were harvested and the number of pods per raceme and of seeds per pod recorded.

Seed yields from the treated and untreated strips were computed on the basis of the seed weight from 10 one-square yard samples harvested from each strip.

FIGURE 1. The Coutts-Evjen Mechanical Tripper.

FIGURE 2. View showing the mechanics of the machine.

FIGURE 3. Close-up of the sponge-covered rollers which rotate at different speeds. The shearing force plus the pressure between the twin rollers causes some of the flowers to trip as the growth passes between them.

FIGURE 4. *Racemes from different treatments of alfalfa flowers:*

A. Tripping followed by self-pollination
B. Tripping followed by cross-pollination
C. Open check
D. Caged check
E. From cage containing honey bees
F. From cage containing leaf-cutter bees
G. From cage containing bumble bees
H. From flowers tripped by mechanical tripper

RESULTS AND DISCUSSION

The Influence of Artificial Self- and Cross-Pollination and of Bees on Seed Setting

In Table 1, the data on tripping, pod and seed setting from the various treatments are summarized.

Cross- and Self-Pollination

The results from Table 1 indicate the importance of tripping followed by cross-pollination as contrasted with self-pollination. Only 31.6 per cent of the selfed flowers formed pods. Moreover, the selfed pods contained an average of only 1.7 seeds per pod. Typical racemes from self-pollinated flowers are illustrated in Figure 4A.

The results of tripping followed by crossing are strikingly different from those of selfing. Pods formed from 74.8 per cent of the treated flowers, and the majority of these pods contained more than three seeds (Table 2).

As can be seen (Figure 4B), the appearance of racemes and pods corresponds with the actual counts of the number of pods set and also of seeds per pod.

These results suggest that very likely most of the pods which had three or more seeds were the result of cross-pollination while those with two or less were the result of self-pollination. This substantiates results obtained by other workers (4, 8, 13). It is likely, therefore, that the number of seeds per pod can be used to determine whether pod setting resulted from cross- or self-pollination.

The Exclusion of Pollinating Insects

When all insects were excluded, only three out of 383 flowers under study were tripped, and pods formed contained only one or two seeds each. One of the flowers that formed a pod, however, must have tripped after the study was discontinued since there was no record of tripping for this flower while the observations were in progress. Needless to say, the effect of automatic tripping under cages is not necessarily related to that which would occur under natural conditions.

The Influence of Honey Bees

Under conditions of this test, honey bees were ineffective trippers (Table 1, Figure 4E). Only 12 of the 473 flowers under study were tripped during the five days that they were exposed to honey bees. Throughout this study honey bees were observed many times in alfalfa fields but were not seen to trip flowers at the time they visited them. Furthermore, pollen-gathering honey bees were not seen in alfalfa fields during the course of this study. Seven of the 12 flowers tripped set pods producing 26 seeds. Twenty-three of these seeds were in three pods on raceme No. 10 shown in Figure 4E. Such a high number of seeds per pod indicates that these pods were very likely the result of cross-pollination. Possibly a honey bee visited an automatically-tripped flower and became covered with pollen unrelated to that of the flowers on raceme 10. Later the bee visited raceme 10, upon which four flowers had tripped automatically and brought

TABLE 1.—THE EFFECT OF DIFFERENT TREATMENTS ON TRIPPING, POD AND SEED SETTING IN ALFALFA

Treatment	Flowers studied			Pod setting			Seed setting			
	Number studied	Number tripped	Tripping in percentage	Total	Percentage of total flowers	Percentage of tripped flowers	Total	Seeds per pod	Seeds per tripped flower	Seeds per total flower
Uncaged check	419	193	46.1	97	23.2	50	426	4.4	2.0	1.02
Caged check	383	3	0.8	3	0.8	100	5	1.7	1.7	0.01
Caged honey bees	473	12	2.5	7	1.5	58	26	3.7	2.2	0.06
Caged bumble bees	420	379	90.2	234	55.7	62	1043	4.5	2.8	2.48
Caged leaf-cutter bees	467	285	61.0	181	38.8	64	807	4.5	2.8	1.73
Selfed	345	345	—	109	31.6	32	185	1.7	0.5	0.54
Crossed	329	329	—	246	74.8	75	1162	4.7	3.5	3.53

TABLE 2.—FREQUENCY DISTRIBUTION OF SEEDS PER POD FROM DIFFERENT TREATMENTS. (TIGER LILY DATA ONLY)

Treatment	Number of seeds per pod										
	1	2	3	4	5	6	7	8	9	10	11
Selfing	35	22	10	1	2	—	—	—	—	—	—
Crossing	4	13	17	10	9	8	12	5	10	5	—
Caged check	1	2	—	—	—	—	—	—	—	—	—
Caged honey bees	3	1	—	—	—	2	—	—	1	—	—
Caged leaf-cutter bees	10	11	12	12	9	6	10	5	8	5	1
Caged bumble bees	9	9	17	13	11	13	9	7	7	2	—
Uncaged check	8	9	6	10	7	10	6	6	2	6	1
Mechanical tripper—											
Once over	9	8	5	—	—	—	—	2	—	—	—
Twice over	10	9	9	2	2	2	—	—	—	—	—
Three times over	22	14	11	5	2	1	2	—	—	—	1

about cross-pollination. An alternative explanation might be that this particular plant was highly self-tripping and self-fertile.

As in the caged check, some of the tagged flowers formed pods after removal of the bees and, after close observation on tripping, had been discontinued. The interesting and probably highly important point is that more automatic tripping occurred in the caged honey bee treatment after the removal of the bees than occurred throughout the entire test in the caged check. As is indicated in Table 3, eight pods resulted from flowers that were tripped after the removal of the honey bees.

TABLE 3.—COMPARISON OF AUTOMATIC TRIPPING OCCURRING IN THE CAGE CHECK (B) AND THE CAGE THAT CONTAINED HONEY BEES (C)

Flowers tripped during 5-day period		Pods forming		Flowers known to have tripped automatically	Total yield of seed from caged area
		During 5 days	Total		
B	3	3	4	4	1.6 grams
C	12	7	15	8	1.5 grams

These findings agree with those of Lesins (9), who reports that nectar-collecting honey bees increase the amount of automatic tripping. Though there was practically no difference in the total amount of seed produced under the two treatments, it may well be that if honey bees had been kept in the cage for a longer period of time, the yield would have been higher. The increase would not have been great, however, because the automatically tripped flowers would have been self-pollinated. A possible explanation of the increase in automatic tripping resulting from the presence of honey bees may be that the removal of nectar causes a loss in turgidity which might bring about more automatic tripping.

The Influence of Leaf-Cutter and Bumble Bees

Since the results with both kinds of bees were in many respects very similar, they will be dealt with together. Bumble and leaf-cutter bees tripped 90.2 per cent and 61.0 per cent of the tagged flowers, respectively.

This difference probably resulted from a difference in the working behaviour of these bees. Bumble bees were actively working at temperatures as low as 50° F., while leaf-cutter bees were rarely active at temperatures below 70° F. Furthermore, it was apparent that leaf-cutter bees were more affected by the caging treatment in that they spent considerably more time trying to escape.

With respect to efficiency of pod and seed setting, there was very little if any difference (Figure 4). Of the flowers tripped by bumble bees, 62 per cent set pods; of those tripped by leaf-cutter bees, 64 per cent set pods. The number of seeds per pod was 4.5 for both. It is evident, therefore, that both leaf-cutter and bumble bees cross-pollinate when they trip alfalfa flowers.

Seed Setting Under Natural Conditions

Of the tagged flowers studied under natural conditions, 46.15 per cent were tripped, compared with only 0.8 per cent in the caged check. Moreover, when the seeds-per-pod data are examined (Tables 1 and 2, and Figure 4) it is evident that most of the pods formed under natural conditions were from flowers that were cross-pollinated when they were tripped. Since wind-pollination can be disregarded (19), pollen-collecting bees must have been responsible for the tripping and cross-pollinating that led to the good seed setting in this field.

The results show that the percentage of tripped flowers setting pods was lower under natural conditions than it was in the cages containing leaf-cutter and bumble bees. One explanation may be that the heavy population of honey bees present on this field was responsible for some automatic tripping. As has been shown, only a very small portion of such flowers would set pods.

TEST WITH THE MECHANICAL TRIPPER

Seed Yields from Strip-Treatments

In Table 4 the results of the tests with the mechanical tripper on the Tiger Lily field are summarized. It is obvious that the use of the tripping machine neither increased nor decreased the amount of seed that was set. Actually, all the machine-treated strips yielded less seed than the check, but in no case was the difference significant.

TABLE 4.—SEED YIELD OF STRIPS TREATED WITH MECHANICAL TRIPPER (TIGER LILY FIELD)

Treatment	Mean yield of ten one-square yard samples (gm.)	S.E.	Treatments compared with check for significance
Check	24.1	2.73	
Once over	21.6	1.91	N.S.
Twice over	20.6	2.14	N.S.
Three times over	22.6	2.54	N.S.
Twice over at different dates	22.5	4.60	N.S.

The results from tests on the Edmonton field (Table 5), likewise, were inconclusive. Only in one case, that of the twice over followed by once over

TABLE 5.—SEED YIELD OF STRIPS TREATED WITH THE MECHANICAL TRIPPER (EDMONTON FIELD)

Treatment	Mean yield of ten one-square yard samples (gm.)	S.E.	Treatments compared for significance with:	
			Check I	Check II
A	4.5	0.54	N.S.	5%
B	4.5	0.87	N.S.	5%
Check	6.5	1.41	N.S.	N.S.
C	6.3	0.55	N.S.	N.S.
D	10.1	0.55	5%	N.S.
E	4.8	0.53	N.S.	N.S.
F	8.2	0.94	N.S.	N.S.
Check II	8.9	0.98	N.S.	N.S.
G	8.6	1.00	N.S.	N.S.

at a later date, was the yield actually higher than the check. The actual increase was not significant when compared with the yield from the check strip closest to it.

It is, therefore, evident that the mechanical tripper under conditions of these tests did not effect greater seed set even when the same area was passed over several times.

Flower Study with the Mechanical Tripper

In Table 6 the results from the tagged-raceme studies with the mechanical tripper are summarized. The machine accomplished very effectively the purpose for which it was designed and built. The percentage tripping was similar to that occurring under natural conditions, but pods formed from flowers tripped by the machine contained only half as many seeds as those formed from natural tripping. There are probably two main reasons for this situation. Firstly, it was observed that the older flowers on a raceme were those most frequently tripped. Studies by Knowles (8) have shown that less pod setting occurs when old flowers are tripped. Secondly, most of the flowers that were tripped were obviously self-pollinated as is indicated by the data on seeds per pod in Table 2. In Figure 4, the striking similarity between racemes from machine tripped and self-pollinated flowers is indicated.

As shown in Table 6, the data from the Edmonton and Tiger Lily fields differ in some respects. In the tests on the Edmonton field, once over with the machine tripped 46.3 per cent of the flowers, of which 29 per cent set pods, while at the Tiger Lily field only 28.2 per cent of the flowers were tripped but of them 47 per cent set pods. The explanation probably is that there was a high concentration of wild bees at Tiger Lily (a random count of bumble bees gave a figure of approximately 4000 per acre during warm sunny conditions). Consequently, there were fewer flowers left to wilt and the machine had to trip more young flowers in the Tiger Lily field. Relatively few bees were present, however, in the Edmonton field and the flowers persisted for a longer time. These older flowers, though they trip more easily, are known to be less fertile (8).

The machine, therefore, tripped effectively but it did not cross-pollinate appreciably. The ultimate result was that under conditions of the test it neither increased nor reduced the yield. It is conceivable that

TABLE 6.—THE EFFECT OF THE MECHANICAL TRIPPER ON TRIPPING, POD AND SEED SETTING OF ALFALFA FLOWERS

Treatment	Flowers studied			Pod setting			Seed setting			
	Number studied	Number tripped	Tripping in percentage	Total	Percentage of total flowers	Percentage of tripped flowers	Total	Seeds per pod	Seeds per tripped flower	Seeds per total flower
Tiger Lily Field—										
Machine										
Once over	195	55	28.2	26	13.3	47	62	2.4	1.1	0.3
Twice over	209	106	50.7	34	16.3	32	76	2.2	0.7	0.4
Three times over	230	145	63.0	70	30.4	48	164	2.3	1.1	0.7
Selfed by toothpick	345	345	—	109	31.6	32	185	1.7	0.5	0.5
Crossed by toothpick	329	329	—	246	74.8	75	1162	4.7	3.5	3.5
Uncaged check	419	193	46.1	97	23.2	50	426	4.4	2.0	1.0
Edmonton Field—										
Machine										
Once over	778	361	46.3	105	13.5	29	193	1.8	0.5	0.3
Selfed by toothpick	296	296	—	131	44.3	44	203	1.6	0.7	0.7
Crossed by toothpick	288	288	—	181	62.8	63	721	4.0	2.5	2.5

if few or no pollinating bees were present, the machine might be of some value. It is questionable, however, whether the seed from machine tripped flowers would be desirable because of the proportion of selfed seed. Such a machine would be valuable for producing seed on self-fertile, non-self-tripping lines, if and when such lines are developed by plant breeders.

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THE ISOLATION OF LADINO CLOVER PLANTS RESISTANT TO *SCLEROTINIA TRIFOLIORUM*[1]

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ABSTRACT

Approximately 57,000 cuttings and seedlings of Ladino clover and white clover were inoculated with *S. trifoliorum* in a greenhouse moist chamber. The inoculum was prepared by growing the fungus on a medium composed of two parts wheat and one part oats.

Plants were selected from screening tests of strains, polycross progenies and clones obtained from uninoculated field plantings. Several partially resistant plants were isolated by means of repeated clonal tests of survivors. Some of these plants transmitted relatively high resistance to their progenies. On the average of five greenhouse tests the F_1 progenies (resistant $\times$ resistant) of one partially resistant clone had 34.9 per cent healthy survivors. In these tests, the average per cent of healthy survivors in the susceptible $\times$ susceptible progenies was 10.3.

Significant differences in survival were demonstrated among various strains of Ladino and white clover.

Difficulties associated with the isolation of resistant material are discussed, together with possible improvements in procedure.

Investigations conducted by Kreitlow (2) indicate that *Sclerotinia trifoliorum* Erikss. may reduce stands of Ladino clover (*Trivolium repens* L.). Since this fungus attacks plants at relatively low temperatures, there are undoubtedly instances when the resulting loss of stand has been attributed to winter injury. Although Sclerotinia crown rot is not the sole cause of winter damage, it may be a major factor under conditions favourable to the organism. If appreciable resistance can be bred into an adapted strain, one of the hazards affecting stands of Ladino will be partially circumvented.

In the interval between 1946 and 1952 an intensive search was made for Ladino clover plants possessing some resistance to *S. trifoliorum*. The results obtained to date are presented and the effectiveness of the procedures used are discussed. A preliminary report was presented by Hanson, Kreitlow, Brown and Trimble (1) at the 1950 meeting of the American Society of Agronomy.

MATERIALS AND METHODS

Inoculations were made in the greenhouse in a moist chamber (3). The chamber consisted of a wooden frame fastened to a bench and covered with clear plastic shower curtain (Parafilm). Sufficient light entered through this curtain to prevent etiolation during the testing period. A humidifier was used to release a fine mist in the chamber at intervals sufficient to keep the plants covered with a film of moisture.

Seedlings and cuttings were used for the inoculation studies. In most tests fifty plants spaced approximately 2 inches apart were established in each flat. When the plants had developed four or five fully expanded

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leaves they were inoculated by placing a liberal quantity of dried grain inoculum around the base of the plants. The grain inoculum was prepared by growing the fungus on a medium composed of two parts wheat and one part oats as described by Kreitlow (4). The same isolates of *S. trifoliorum* were used in all experiments.

Inoculated plants were kept in the moist chamber for 7–14 days at a temperature of 15–20° C. When difficulty was experienced in maintaining this temperature, the inoculated plants were placed in a constant temperature room illuminated with Mazda lamps. The plants were watered often enough to keep the inoculum moist.

Some 20,000 seedlings representing various sources of *Trifolium repens* L. and approximately 26,000 cuttings from 500 clones were screened in greenhouse inoculation tests. Plants isolated in this phase of the program were used to produce the F_1 generation, of which approximately 11,000 seedlings were inoculated.

Healthy plants selected from seedling tests as well as individual plants obtained from uninoculated field nurseries were propagated vegetatively for testing. When a large number of clones were available, they were generally compared in a single replication of five plants each. The more promising clones were then screened in replicated trials, and reselections subsequently tested two or three times.

At the termination of each experiment the plants were classified as healthy, damaged or dead. Plants that were subjected to two successive inoculations were interpreted on the basis of the percentage survivors (healthy + damaged) while those subjected to a single inoculation were interpreted on the basis of the percentage of healthy survivors only. The behaviour of identical clones, or a common commercial entry in seedling tests, served as a basis for comparing different experiments. Seedling and advanced clonal tests were analysed statistically, the data being transformed whenever necessary (5).

EXPERIMENTAL RESULTS

Observations on inoculation technique

In spite of precautions taken to ensure uniformity the variation within and between tests was considerable, and coefficients of variation in excess of 80 per cent were obtained in some trials. The variation within tests could be partially compensated by additional replication, but the most effective procedure proved to be daily randomization of the flats in the chamber. In later trials where this practice was followed the coefficients of variation were frequently reduced to 30 to 40 per cent.

The possibility exists that heavy applications of inoculated ground grain may have a deleterious effect on plants, exclusive of the action of the fungus. Therefore, an experiment was conducted in which the effect of uninoculated and inoculated ground grain was compared. Sufficient uninoculated grain was applied to completely cover the crown, while the inoculated grain was applied as follows: (*a*) heavy application (amount comparable to application of uninoculated grain); (*b*) light application (half amount used in, *a*, applied to crown), and (*c*) broadcast (amount used in, *b*, scattered over moist plants). None of the plants treated with ground grain was

killed while the inoculated plants were severely damaged (heavy application —90 per cent killed; light application—50 per cent killed; broadcast—60 per cent killed).

The low light intensity in the chamber may exert an indirect effect on the results of inoculation tests. If Ladino clover plants exhibit a differential tolerance to reduced light some clones may become weakened in the chamber and may be more subject to attack by *S. trifoliorum*. To investigate this possibility inoculated and uninoculated plants of five clones and commercial Ladino seedlings were placed in three temperature control chambers at light intensities of 50-, 300- and 600-foot candles. At the termination of the inoculation period it was concluded that light intensity had not exerted an appreciable effect on survival.

The effectiveness of the inoculum was demonstrated in field experiments. Sixteen clones which exhibited differential survival in greenhouse tests were established in clonal plots and inoculated in the fall with dried grain inoculum. In this experiment the close relationship of vigour to survival was clearly demonstrated in both the inoculated plots and uninoculated checks. Partially resistant clones not only had the largest percentage of survivors, but also exhibited more uniform infection in the two inoculated replications. One susceptible clone had an average of 30 per cent survivors, but this percentage consisted of 8 per cent survival in the first replication and 52 per cent in the second. The average survival of the resistant clones was 29 per cent (range in averages, 44 to 10 per cent) while the average of the susceptible entries was 15 per cent (range in averages, 30 to 2 per cent). These results suggest that at least some of the clones selected from greenhouse tests will also exhibit good survival in field inoculation tests. In a second field trial the survival of commercial Ladino seedlings was comparable to that obtained in the greenhouse.

Strain and progeny tests

Survivors obtained from seedling tests of strains and polycross progenies provided important source material for additional screening trials. The strains and progenies compared in these experiments exhibited marked differences in percentage survival, a feature which can be illustrated by the results from a replicated strain test of Ladino clover from eleven sources and white clover from seven sources (Table 1). In this experiment two inoculations were made.

All the white clover strains except the one from Louisiana had a significantly higher percentage of survivors than the commercial Ladino check. On the other hand, only one strain of Ladino clover, F.C. 23087 from Italy, possessed a significantly greater percentage of survivors.

In general, the white clover strains had a higher percentage of survivors than Ladino clover but within the two groups there was an appreciable range in survival. The data indicate that some clover strains may provide excellent source material for resistance to *S. trifoliorum*. There is also some indication that the survival of white clover and Ladino clover may be associated with the severity of Sclerotinia crown rot in the region where the strains were grown. Caution should be exercised in interpreting data of this type, however, for it is extremely unlikely that the samples tested can be considered as truly representative of any particular strain.

TABLE 1.—THE SURVIVAL OF LADINO AND WHITE CLOVER SEEDLINGS FROM VARIOUS SOURCES FOLLOWING TWO INOCULATIONS WITH *Sclerotinia trifoliorum*.

Source	Number of Plants	% Healthy	% Damaged	% Survival
Ladino Clover				
FC 23035 California	294	3.1	2.4	5.5
FC 23036 California	462	8.6	4.5	13.1
FC 23037 California	462	4.3	3.0	7.3
FC 23038 California	462	6.9	4.3	11.2
FC 23039 California	462	5.2	5.2	10.4
FC 23056 Oregon	462	8.0	4.5	12.5
FC 23060 Oregon	462	7.6	3.5	11.1
FC 23067 Montana	462	7.1	5.6	12.7
FC 23086 Italy	294	8.8	3.1	11.9
FC 23087 Italy	294	15.0	6.5	21.5*
FC 23088 Italy	448	10.0	4.5	14.5
Commercial Ladino	1345	5.6	2.8	8.4
White Clover				
PEI 22075 Kent	210	25.2	5.7	30.9**
FC 22889 Louisiana	462	7.8	4.1	11.9
FC 30652 Kentucky	462	9.1	6.5	15.6*
FC 22461 Wisconsin	462	9.7	8.4	18.1*
PEI 133330 New Zealand	294	11.6	6.8	18.4*
Scott's Comm.	462	13.4	5.0	18.4*
Past. Lab. Composite	462	9.5	9.3	18.8*

* Significant at the 5 per cent level.
** Significant at the 1 per cent level, when compared with commercial Ladino.

Clonal Tests

Preliminary screening tests in which clones were represented by a small number of cuttings, often arranged in a single replication, were evaluated entirely by inspection. As a consequence the agreement between preliminary tests was often very poor.

The scope of the clonal testing may be illustrated with the 105 entries included in the 1947 Ladino polycross nursery.

(*a*) *First test*—105 clones examined in one replication of five plants each. Twenty-eight clones appeared to warrant further testing.

(*b*) *Second test*—40 clones (28 clones from first test plus 12 susceptible checks) planted in two replications of five plants each. Eighteen clones selected and included in three additional trials.

(*c*) *Third test*—24 clones (18 clones from second test plus six susceptible checks) planted in four replications of five plants each. Two clones classified as outstanding and 14 clones as moderately resistant.

(*d*) The inoculation was not satisfactory in the fourth test. In the fifth test which consisted of four replications of 25 plants each, three clones had a significantly higher percentage of healthy survivors than the checks. In the third test one of these three clones had been classed as outstanding and the other two as moderately resistant. There was no relationship between the average response of the clones in the first and second tests ($r = 0.04$). The correlation coefficient for the average of the second and third tests, with test five, was positive and highly significant ($r = 0.59$).

TABLE 2.—THE AVERAGE PERCENTAGE OF HEALTHY SURVIVORS IN AN ADVANCED TEST OF 24 CLONES SELECTED FROM AN INOCULATION TEST OF POLYCROSS PROGENIES

Clones	Number of Plants	% Healthy	Clones	Number of Plants	% Healthy
1	45	57.5**	13	43	46.1
2	41	24.2	14	44	52.7
3	49	40.9	15	39	31.0
4	44	57.5**	16	50	32.9
5	43	59.8**	17	49	62.4**
6	44	61.4**	18	46	58.7**
7	36	48.4	19	43	51.0
8	43	27.8	20	45	75.5**
9	50	59.6**	21	44	68.2**
10	46	34.8	22	45	29.0
11	44	52.3	23	47	38.0
12	47	49.0	24	47	29.7
Check	43	16.3			

** Significant at the 1 per cent level when compared with clonal check.

TABLE 3.—NUMBER OF PROMISING (PARTIALLY-RESISTANT) CLONES SELECTED FROM LADINO CLOVER OF FOUR GENERAL SOURCES

Source	Number of Clones	Number of Promising Selections
(1) *Survivors from:*		
(*a*) Strain test (seedlings)	79	7
(*b*) 1947 polycross (seedlings)	24	7
(2) *Clones from*		
(*a*) 1946 progeny test	228	4
(*b*) 1947 polycross	105	3

The results from an advanced inoculation test of 24 clones is presented in Table 2, to illustrate the range in response among clones selected from previous experiments. The differences between nine of the clones and the susceptible clonal check were highly significant for percentage of healthy survivors.

The clones selected at the conclusion of any one trial included only those individuals which possessed a consistently high percentage of healthy survivors in all replications. This procedure seemed justified in view of the objective of these experiments, namely, to isolate clones which possess appreciable resistance to *S. trifoliorum*.

The plant material tested and the number of clones selected are given in Table 3. As expected, survivors isolated from seedling tests tended to yield a higher percentage of promising selections than clones obtained directly from uninoculated field plantings.

F_1 *Progeny Tests*

Clones that showed some resistance to *S. trifoliorum* together with clones that had received low survival ratings were established in the greenhouse for diallel crossing. Fourteen partially resistant and 12 susceptible

TABLE 4.—PERCENTAGE OF HEALTHY SURVIVORS IN FIVE INOCULATION TESTS OF SINGLE CROSS PROGENIES (TESTS ADJUSTED ON BASIS OF SIX PER CENT HEALTHY SURVIVORS IN COMMERCIAL LADINO CLOVER CHECK)

Source	Total Number of plants	Test					Average per cent
		1	2	3	4	5	
R × R	4005	28.5	26.5	32.0	17.7	17.1	24.4
R × S	2525	21.5	18.5	21.0	8.2	16.5	17.1
S × S	3230	12.0	4.0	13.0	11.0	11.7	10.3
R × R*	775	41.5	36.0	34.0	29.6	33.3	34.9
R × R**	947	26.0	20.5	28.0	17.7	10.8	20.6

* Crosses involving parent 40–9.
** Parent 28.

clones were used. The progenies from these crosses were inoculated in five separate experiments. The number of progenies in these experiments varied from 81 to 121 arranged in four replications of five plants each. In order to facilitate comparisons between trials, certain single cross progenies together with commercial checks were common to all experiments.

The differences among progenies were highly significant in each of the five experiments, while the differences between the averages of the resistant × resistant and sucseptible × susceptible progenies were highly significant in tests one through four, but not significant in the fifth test. The average of the resistant × susceptible progenies did not differ significantly from the average of the resistant × resistant progenies except in the fourth test. The averages of the susceptible × susceptible progenies were significantly lower in survival than the resistant × susceptible progenies in tests one, two and four.

Some of the variation among tests can be attributed to the fact that the single cross progenies representing the three categories were not of identical parentage in all five tests.

The magnitude of the differences encountered among F_1 progenies derived from different parents but belonging to the same category with respect to resistance is illustrated by clones 28 and 40–9. On the average of the five tests, crosses involving 40–9 possessed 34.9 per cent healthy survivors, while all of the resistant × resistant progenies averaged 24.4 per cent. On the other hand, the average performance of clone 28 was consistently lower than the average of the resistant × resistant progenies. In the fifth test the performance of clone 28 was similar to that of the susceptible × susceptible progenies.

On the basis of the five tests the average percentage of healthy survivors in the resistant × susceptible progenies was approximately midway between that of the resistant × resistant and the susceptible × susceptible progenies. It will be observed that in general the percentage of healthy survivors in the susceptible × susceptible category was slightly higher than that of the commercial check. Since several of the susceptible clones had survived one or two inoculations, it is probable that these plants possessed at least a limited amount of resistance to *S. trifoliorum*.

DISCUSSION AND CONCLUSIONS

The results obtained in this investigation indicate the practicability of selecting Ladino clover plants capable of transmitting a relatively high degree of Sclerotinia crown rot resistance to their progenies. The evidence available suggests that resistance is governed by several genetic factors. This implies that it may be feasible to isolate plants which have a higher level of resistance than the best plants found in this study.

An obvious source of variation in greenhouse inoculation trials is associated with differences in vigour that occur among and within the entries in seedling and clonal tests. This is especially serious in clonal tests where it often becomes increasingly difficult to propagate some clones. The accumulation of virus diseases may be a factor contributing to the decline in vigour which accompanies successive multiplications. Under these circumstances it is possible that certain clones could be discarded during the screening process even though they possess some resistance to *S. trifoliorum*.

While the method of repeated clonal tests adhered to in this study has been effective in isolating some promising material, it may not be the most efficient approach to the problem. The results suggest that the effectiveness of selection would have been increased materially if progeny tests had been used in conjunction with clonal evaluation. The scope of the preliminary screening tests could be increased by inoculating various strains of Ladino clover in the field, and increasing the survivors vegetatively for greenhouse testing. The better clones isolated in this phase of the program could be intercrossed either in the greenhouse or under isolation in the field. The progenies would then be tested in the greenhouse and survivors selected for clonal testing to repeat the cycle.

ACKNOWLEDGMENTS

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THE EFFECT OF RESIDUAL PENICILLIN IN MILK ON THE DYE REDUCTION TESTS FOR QUALITY[1]

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ABSTRACT

Confirming earlier findings, results by the methylene blue reduction test were frequently found to be affected to an appreciable degree by small concentrations (0.05 and 0.5 units/ml.) of penicillin added to raw milks. Because of its shorter incubation period, results by the resazurin "triple reading" test were affected to a much lesser extent. The report that resazurin results were affected more when milks containing penicillin were refrigerated overnight was investigated but the effect was found to be too slight to materially affect the results.

The dye reduction tests, using methylene blue and resazurin, are widely used in assessing the bacteriological quality of raw milk (1). These tests reflect the metabolic activity of the organisms present and growing in the milk during incubation at 37° C. If a significant percentage of these organisms is inhibited by residual penicillin or other antibiotics used to treat mastitis, the reduction time may be prolonged and the milk appear to be of better quality than it really is.

Johns and Katznelson (5) reported that concentrations of penicillin in milk as low as 0.05 units/ml. could delay the reduction of methylene blue appreciably. With the "triple reading" resazurin test, however, where the Munsell P7/4 end-point is generally reached in approximately half the time taken to decolourize methylene blue, reduction time was rarely prolonged significantly. Hunter (3) in New Zealand reported a variable effect on methylene blue reduction time when penicillin was added to milk. Ruehe (7) described a small series of tests where the penicillin generally had little or no effect. These varied findings probably reflect differences in the flora of the milks studied. Since this paper was prepared, Rowlands (6) has reported that concentrations of penicillin of 0.25, 0.5 and 1 units/ml. increased methylene blue reduction time from 0 to >3 hours on 32 samples. The effect was generally as marked with 0.25 units as with higher concentrations, but 0.1 units generally had no effect.

No other published results of studies of resazurin have been seen but it has recently been reported (2) that where penicillin is added to the test milks before overnight refrigeration, instead of immediately prior to testing (5), the reduction of resazurin may be delayed to a significant degree. To check on this point, and to supplement the previous work of the authors, two series of studies have recently been carried out.

EXPERIMENTAL

A. Effect on Reduction Time of Stage at which Penicillin is Added to Milk

Each week for 4 weeks, 12 samples of producers' milks were collected at local pasteurizing plants. Eleven 10 ml. portions were dispensed into

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sterile tubes; one was laboratory pasteurized at 62.8° C. for 30 minutes. Five tubes containing methylene blue (1) were treated as follows:—

A — 0.5 units/ml. penicillin added at once
B — 0.05 " " " " " "
C — 0.5 " " " " next morning
D — 0.05 " " " " " "
E — Control—No " "

The remaining 5 tubes, containing resazurin (1), received similar treatments. All 10 tubes of milk, containing either resazurin or methylene blue, were held overnight at 2° C. and incubation started early next day. Resazurin colour numbers were recorded using the following system devised by Johns and Howson (4):

Shade	*Munsell Notation*	*Resazurin Colour No.*
Initial Blue	PB 7/4	0
Blue—Lilac	PBP 7/5.5	4
Mauve	P 7/4	8
Mauve—Pink	PRP 7/8	12
Pink		16
White		24

Readings were made at 30-minute intervals for the first 3 hours, then every hour. Methylene blue tubes were inspected at 30-minute intervals and more frequently when necessary.

RESULTS AND DISCUSSION

The average values obtained from the 48 samples tested (Table 1) show that the reduction time of methylene blue is much the same, whether the penicillin is added before or after overnight refrigeration. Both concentrations retarded reduction appreciably compared with the control.

TABLE 1.—AVERAGE REDUCTION TIMES AND COLOUR NUMBERS OF 48 SAMPLES AS INFLUENCED BY PENICILLIN CONCENTRATION IN MILK (MAR. 7–27, 1952)

	0.5 units/ml.		0.05 units/ml.		Control
	A	C	B	D	E
Methylene Blue Reduction Time (hr.)	6.6	6.7	6.2	6.1	5.0
Resazurin Colour No. at 1 hr.	6.3	6.4	6.1	6.5	6.5
2 "	8.6	8.9	8.9	9.7	9.8
3 "	10.6	11.0	11.6	12.8	13.8
4 "	12.5	13.0	14.1	14.7	17.1
5 "	14.2	14.8	16.2	16.5	19.7
6 "	16.0	16.3	18.1	18.5	21.8

A and B—Penicillin added before overnight refrigeration.
C and D— " " after "

The results with resazurin are more complex. The average colour number was slightly lower when the higher concentration of penicillin was added the day before; with the lower concentration this difference was somewhat larger. This is more evident in Figures 1 and 2 where the data are presented graphically.

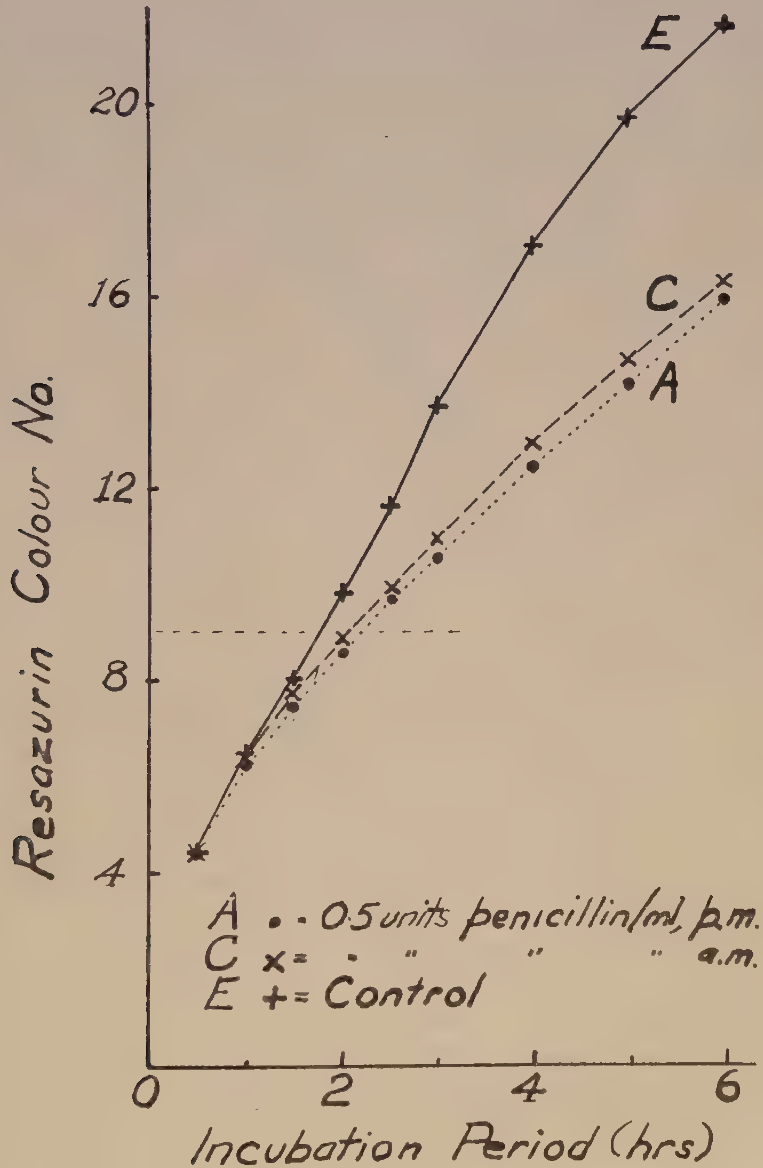

FIGURE 1. Effect of time of adding penicillin (0.5 units/ml.) on resazurin colour number.

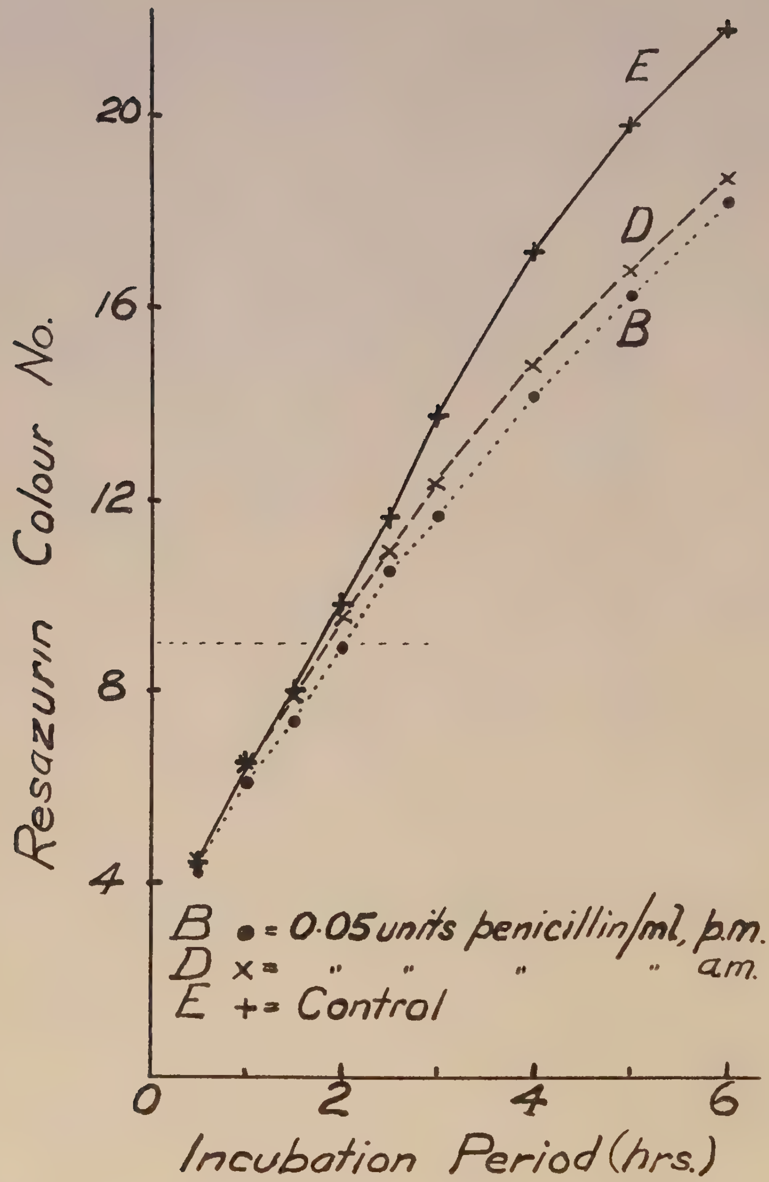

FIGURE 2. Effect of time of adding penicillin (0.05 units/ml.) on resazurin colour number.

An important consideration is to what extent the stage at which the penicillin is added affects the time required to pass the P7/4 end-point (Colour No. 8). Figure 1 indicates that where 0.5 units penicillin were added before overnight storage the average time required to reach the end-point was delayed by 10 minutes compared with the tubes where penicillin was added next morning. Figure 2 shows that, with 0.05 units, this difference increased to 12 minutes.

Another way of assessing the influences of the time of adding penicillin is to tabulate the distribution of the samples according to the time required to reduce each dye to the designated end-point. Table 2 shows the percentages of samples which have reduced each of the dyes at specific intervals. These data again show that the time of adding the penicillin had little influence upon the rate of reduction with either test.

TABLE 2.—EFFECT OF TIME OF ADDING PENICILLIN ON REDUCTION TIMES OF 48 MILK SAMPLES. (Figures represent cumulative totals of percentages of samples reduced at each period.)

Units Penicillin/ml.	0.5		0.05		0
	A[1]	C	B	D	E
	%	%	%	%	%
Resazurin Reduction Time (hr.)					
1	21	21	21	25	23
2	33	33	46	52	54
3	54	56	60	63	69
4	63	65	73	73	79
5	73	75	85	85	94
6	83	83	90	88	98
Methylene Blue Reduction Time (hr.)					
1	4	4	4	4	4
2	8	8	10	10	10
3	10	13	17	17	17
4	21	21	25	25	38
5	23	23	35	35	56
6	40	39	50	47	71
7	50	47	56	56	88
8	60	65	71	75	96

[1] A and B, Penicillin added before overnight refrigeration.
C and D, " " after " "

B. *Relative Sensitivity of Resazurin and Methylene Blue Reduction Tests to Penicillin in Milk*

To supplement the data obtained in the preceding section two additional batches of 20 samples each were treated similarly, except that all tubes were set up with penicillin added before overnight storage. The results from these 40 samples, combined with those from the previous 48, are presented in Figure 3.

In these studies penicillin appears to have affected the reduction of resazurin during the first 3 hours of incubation to a greater extent than it did in the previous studies (5). This is more evident with the stronger concentration of penicillin (0.5 units/ml.), where the effect is generally more than double that of the weaker concentration. Comparing the resazurin data with those for methylene blue, it is clearly evident that the latter are much more seriously affected. Furthermore, the weaker concentration of penicillin (0.05 units/ml.) causes a much greater inhibition here than with resazurin. Finally, in contrast to resazurin, the lag in methylene blue reduction becomes more marked as the incubation period is extended. Since the resazurin test is normally terminated after 3 hours, as against 6 hours or more for the methylene blue test, this gives the former test a

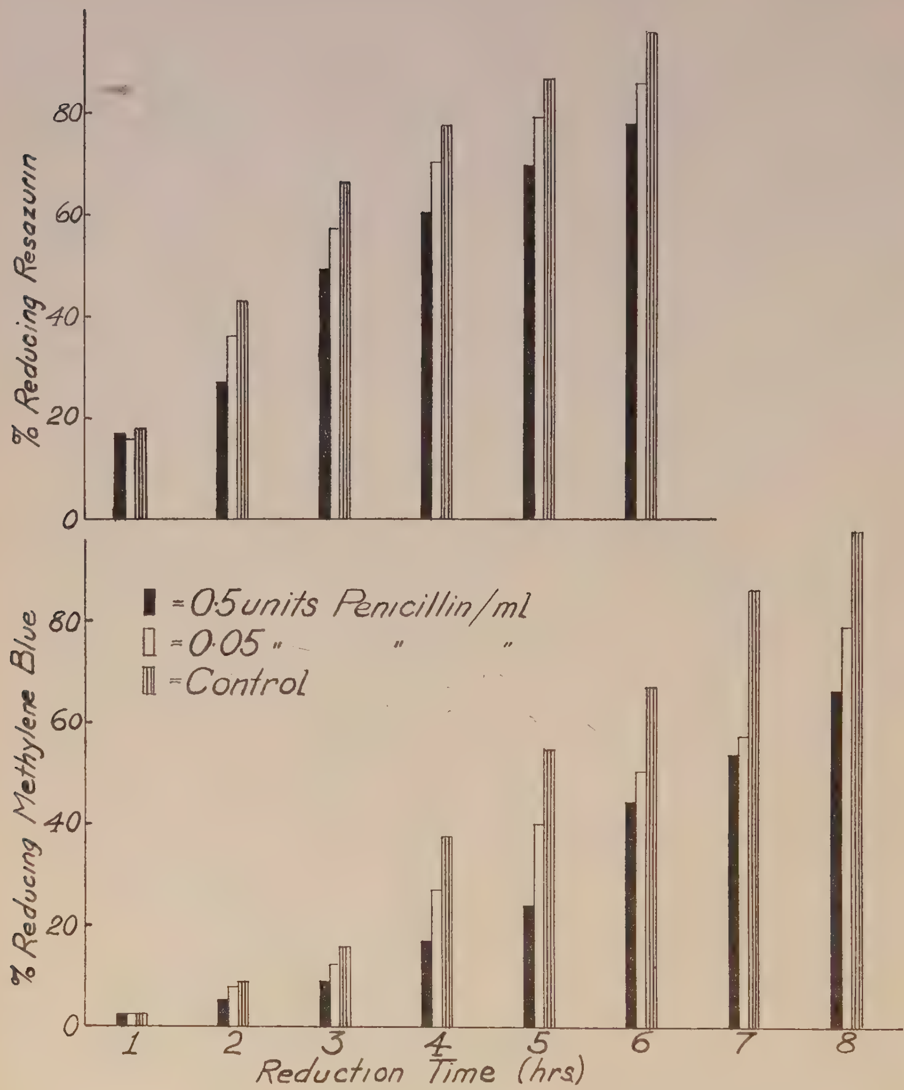

FIGURE 3. Effect of added penicillin on reduction time as indicated by cumulative percentages of 88 milk samples reduced after each hour of incubation.

definite advantage in that the resazurin test results are less likely to be distorted by residual penicillin in the milk.

That the effect of penicillin on dye reduction becomes greater as the incubation period is extended is clearly evident from the data on resazurin colour numbers in Figures 1 and 2. At the resazurin end-point (No. 9) the effect is quite small (16–25 minutes); at the full pink colour (No. 16), it is over 2 hours, and if the curves are extrapolated to the point of complete decolorization (No. 24 or methylene blue end-point) the effect is still greater. This serves to explain why penicillin affects the results by the methylene blue reduction test to a much greater degree than it does those by the resazurin triple reading test.

TABLE 3.—ILLUSTRATING DIFFERENCES IN SENSITIVITY OF MILK FLORA TO PENICILLIN

Sample	Penicillin Conc'n units/ml.	Resazurin Colour Number Readings at (hr.)						Meth. Blue R.T. (hr.)
		1	2	3	4	5	6	
OD78	0.5	5	7	*10*	11	11	12	8.5
	0.05	5	8	*10*	11	11	12	8.5
	0	5	*12*	23	24	24	24	3
PD24	0.5	3	7	8	8	*18*	24	6
	0.05	3	7	*9*	16	21	24	5
	0	3	7	*9*	16	23	24	5
OD172	0.5	5	7	*9*	11	18	24	6.25
	0.05	5	7	*9*	11	19	24	6
	0	5	7	*9*	11	19	24	6

As might be expected, individual milks show striking differences in susceptibility to penicillin. These differences are illustrated by the data in Table 3. Sample OD78 showed extreme sensitivity, PD 24 was affected only at the higher concentration, while OD172 was unaffected.

SUMMARY

The studies reported here indicate that (1) the time at which penicillin is added to milk has little effect upon the results of either dye reduction test; (2) the retarding influence of penicillin increases progressively as reduction time is prolonged and (3) resazurin test results are distorted to a lesser degree than are those by the methylene blue test.

ACKNOWLEDGMENT

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THE APPROACH METHOD OF BARLEY HYBRIDIZATION[1]

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ABSTRACT

The approach method of barley hybridization is described. The general procedure is to enclose the male and female (emasculated) spikes under one enclosure and allow the pollen from the male spike to fertilize the florets on the emasculated spike. Greenhouse and field procedures are outlined in detail. Seed sets of 70-90 per cent were obtained regularly and with a minimum of effort.

There is a technique in cereal hybridization which has been designated "the approach method". The general procedure is to enclose the male and female spikes under one enclosure, such as a glassine bag, and allow the pollen from the male spike to fertilize the florets on the female spike. The first reference to the approach method for hybridization of cereals was published by Jelinek in Germany in 1918 (1). A variation of the method was described by Rosenquist in 1927 (4). He stated that fair results had been obtained with barley. Pope described a similar method for barley in 1933 (2) and presented further details in 1944 (3). The approach method for barley hybridization has been used most successfully at the Cereal Division, Central Experimental Farm, Ottawa. The techniques employed vary considerably from those hitherto described but are somewhat like those which have been used with wheat, at Ottawa, for several years.

One does not necessarily follow the same details of procedure when crossing in the greenhouse and in the field. Thus, the techniques will be described for each set of conditions.

PROCEDURE

Greenhouse

It is preferable to grow the plants in a cool temperature which will allow the spikes to protrude from the boot before flowering. A temperature of 40° F., or slightly above, proved satisfactory. The procedure is as follows:

1. Choose a spike for the female parent in which there is no danger of free pollen being present. Remove the florets in the lateral spikelets; also remove the florets in the basal central spikelets, if they are too far advanced in maturity, and the florets in the tip central spikelets if they are too immature to be emasculated. However, if one wishes to utilize all florets on the spike, they may be emasculated through careful manipulation (*Figure 1*). Clip off approximately one-third of the lemma and palea of each floret, thus leaving the male and female organs exposed. Remove the stamens from each floret. It is preferable to use spikes in which the more advanced female organs are still immature but which could be pollinated, possibly in a few hours, or in a day or two. This is illustrated in Figure 1. Most of the anthers in the two spikes on the left were greenish-yellow in

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FIGURE 1. Preparation of the female spikes: *left,* lateral florets removed; *centre,* no florets removed, all emasculated; *right,* spike emasculated 6 days, too old for pollination.

FIGURE 2. Preparation of the male spikes: *left*, lemmas clipped and anthers exposed; *right*, awns clipped but not lemmas.

Figure 3. Male spike ready for bagging with female. Culm placed in water and moved to location of female.

CONCLUSIONS

Seed sets of 70-90 per cent may be obtained regularly when the above procedures are followed in barley hybridization. Upon occasions, 100 per cent seed set may be obtained. The method is time-saving and easy, and fewer plants are required for crossing. It is favoured particularly for greenhouse hybridization.

ACKNOWLEDGMENTS

The painstaking assistance of S. Symko and D. McNaughton, in helping to perfect these techniques, is acknowledged.

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